



**SURAJO ISAH ALIYU**

**THE FOLIAR APPLICATION OF AMINO ACID ENRICHED  
FERTILIZER IMPROVES DROUGHT TOLERANCE IN  
COMMON BEAN (*Phaseolus vulgaris* L.)**

**LAVRAS -MG  
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Dissertation for master's qualification presented to the Federal University of Lavras as part of the requirement of the postgraduate Program in Agronomy/ Plant Physiology.

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**A APLICAÇÃO FOLIAR DE FERTILIZANTE ENRIQUECIDO COM  
AMINOÁCIDOS MELHORA A TOLERÂNCIA À SECA EM FEIJÃO COMUM  
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## **DEDICATION**

I dedicated this research work to Almighty ALLAH who gives me the opportunity to achieve this remarkable academic success.

I also dedicated it to Abdulkarim Bello Norde for his notable contribution to this success

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## RESUMO

Com a crescente ameaça das mudanças climáticas e da escassez de água, há uma necessidade premente de aumentar a produtividade das culturas e a eficiência do uso da água. O feijão comum (*Phaseolus vulgaris* L.) é uma fonte alimentar vital devido ao seu valor nutricional, o que torna importante melhorar a sua resiliência a deficiências hídricas. Este estudo investigou os efeitos da aplicação foliar de um fertilizante orgânico como fonte de nitrogênio (N) na mitigação das consequências negativas do déficit hídrico e no aumento da produtividade de grãos do feijoeiro. Nossa hipótese é que a aplicação foliar de fertilizante orgânico como fonte de N para o feijoeiro pode aumentar a tolerância a deficiências hídricas e melhorar a produtividade de grãos nas culturas, melhorando o metabolismo de N nessas plantas. O experimento empregou uma configuração de casa de vegetação com dois fatores: disponibilidade de água (bem irrigada e retenção de água) e adubação foliar (com e sem). A variedade de feijão BRSMG MARTE foi submetida à aplicação foliar de adubo orgânico (Vislumbre®; AgroPrime, Brasil) no estádio V4. Sob condições de déficit hídrico, as plantas tratadas com fertilizante orgânico exibiram condutância estomática e transpiração reduzidas, indicando maior eficiência no uso da água. Além disso, elas mantiveram um maior teor de água relativa e eficiência intrínseca do uso da água do que as plantas não tratadas. No entanto, as plantas irrigadas sem fertilizantes orgânicos apresentaram maior fotossíntese. A aplicação de fertilizante orgânico levou a concentrações foliares elevadas de aminoácidos e prolina sob condições de estresse máximo e aumento do acúmulo de amido durante a reidratação. Embora o acúmulo de proteína tenha sido reduzido em plantas tratadas com fertilizante orgânico sob condições de déficit hídrico, elas demonstraram aumento na absorção de amônio. A atividade da ascorbato peroxidase foi aumentada em condições de estresse máximo, enquanto a atividade da superóxido dismutase foi maior em plantas irrigadas com fertilizante orgânico e plantas com deficiência hídrica não tratada. A atividade da catalase foi elevada em plantas não tratadas e com deficiência de água sob condições de estresse máximo. Notavelmente, as plantas tratadas com fertilizantes orgânicos sob condições de déficit hídrico exibiram números de grãos aumentados, indicando maior tolerância à seca. No entanto, eles apresentaram menor peso de sementes e menos vagens do que as plantas irrigadas sem tratamento com fertilizante orgânico. Esses achados destacam o potencial da aplicação foliar de fertilizante orgânico para aumentar a tolerância ao déficit hídrico e a produtividade de grãos em feijoeiro comum.

Palavras- Chave: Estresse hídrico. Tolerância. Crescimento. *Phaseolus vulgaris*.

## ABSTRACT

With the increasing threat of climate change and water scarcity, there is a pressing need to enhance crop productivity and water-use efficiency. Common bean (*Phaseolus vulgaris* L.) is a vital food source due to its nutritional value, which makes it important to improve its resilience to water deficits. This study investigated the effects of foliar application of an organic fertilizer as a nitrogen (N) source on mitigating the negative consequences of water deficit stress and enhancing grain yield in common bean. We hypothesized that foliar application of mineral fertilizer as an N source to common bean could enhance tolerance to water deficits and improve grain yields in crops by improving N metabolism in these plants. The experiment employed a greenhouse setup with two factors: water availability (well-watered and water-withholding) and foliar fertilization (with and without). The common bean variety BRSMG MARTE was subjected to foliar application of mineral fertilizer (Vislumbre®; AgroPrime, Brazil) at the V4 stage. Under water deficit conditions, mineral fertilizer-treated plants exhibited reduced stomatal conductance and transpiration, indicating improved water-use efficiency. Additionally, they maintained a higher relative water content and intrinsic water use efficiency than non-treated plants. However, plants irrigated without mineral fertilizers displayed increased photosynthesis. Mineral fertilizer application led to elevated leaf concentrations of amino acids and proline under maximum stress conditions, and enhanced starch accumulation during rehydration. Although protein accumulation was reduced in mineral fertilizer-treated plants under water deficit conditions, they demonstrated increased ammonium uptake. The activity of ascorbate peroxidase was enhanced under maximum stress conditions, whereas superoxide dismutase activity was higher in irrigated plants with mineral fertilizer and untreated water-deficit plants. Catalase activity was elevated in untreated, water-deficient plants under maximum stress conditions. Notably, mineral fertilizer-treated plants under water-deficit conditions exhibited increased grain numbers, indicating improved drought tolerance. However, they displayed lower seed weights and fewer pods than irrigated plants without organic fertilizer treatment. These findings highlight the potential of foliar application of mineral fertilizer to enhance water deficit tolerance and grain yield in common bean.

Keywords: Water stress. Tolerance. Growth. *Phaseolus vulgaris*.



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## 1.INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) has become an essential grain from a legume with global economic and social importance, being the main source of plant-based protein for humans in Brazil, and some parts of Africa (FAOSTAT, 2021). In order to meet sustainable food production that can satisfy the rising global population's demand, which is expected to reach 9.8 billion people by the year 2050, sustainable food systems are required (NCHANJI; LUTOMIA, 2021). The common bean is the world's most significant edible legume (DUARTE SANTOS; BADIALE FURLONG, 2021). It performs best in moderate growing temperatures (>10 °C and <30 °C) with about 400 mm of precipitation during the growing season. The crop is found in both temperate and tropical growing regions. Common bean popularity originates due to the fact that it is relatively easy to produce, a flavorful and good source of nutrition as it contains approximately 22% protein, several macros, and micronutrients, complex carbohydrates (62%), soluble fiber (15%), it is an important source of folate (MYERS; KMIECIK 2017).

The world's harvested area for dry common beans was 35,920,593 million ha, and their combined output was 27,715,023.7 Mg (FAOSTAT 2021). Presently, there are ten world-leading producing countries: India, Brazil, Myanmar, the United Republic of Tanzania, China, Mexico, the United States, Uganda, Argentina, and Kenya accounting for (61%) of the total production (FAOSTAT, 2021). Brazil, which produces 2.9 Mt and has an average productivity of 1.1 t/ha, is the second-largest producer, coming in behind India (6.1 Mt) and Myanmar (2.4 Mt) been the third (FAOSTAT, 2021). It is a vital food source for the Brazilian population, especially in low-income groups, where it is the primary source of protein (HAYTOWITZ et al., 2018). Due to its wide edaphoclimatic adaptation, in Brazil, the common bean takes part in most production systems of small and medium farmers, being used for household consumption and income generation.

However, different abiotic factors, such as cold, salinity, drought and flooding, have a significant impact on the growth development of the common bean and legume crops (DESOKY et al., 2021). Among them, drought stress is the most challenging issue to agricultural productivity and has a pronounced negative effect on plant growth and development, resulting in damages to productivity, making it difficult to maintain a sustainable agricultural system worldwide (AZEEM et al., 2022; MA et al., 2019).

Earlier studies have reported that water deficit triggered variety of harmful morphological, physiological, and biochemical processes that could seriously disrupt plant

metabolism (SHAKEEL et al., 2011; SANCHEZ-REINOSO; LIGARRETO-MORENO; RESTREPO-DIAZ, 2018). One of these impacts is the significant oxidative stress that the excessive formation of reactive oxygen species (ROS) causes to protein, and lipids (SOFY et al., 2021).

According to GAO et al., (2016), abiotic factors like water stress reduce photosynthesis, due to photochemical limitations caused by limiting the production of NADPH and ATP, as by lowering stomatal conductance, inhibiting CO<sub>2</sub> availability to carboxylation through ribulose 1-5 biphosphate carboxylase/oxygenase (Rubisco) (LIU et al., 2011 ; XU et al., 2020). As a consequence, beans' photosynthetic rates limitations during droughts slows or prevents the buildup of biomass and plant growth (POLANIA et al., 2022).

According to RASTI SANI et al., (2018) common bean is a very sensitive vegetable crop to drought stress, and earlier studies revealed that common bean growth, seed yield, seed quality, physiological and biochemical processes were severely impacted by drought stress. It is known that any change in the relative water content of the leaves, particularly during the flowering stage, may cause a considerable reduction in the production and quality (BESHIR; BUECKERT; TAR'AN, 2016; SALEH et al., 2018). GRAHAM; RANALLI (1997) reported that common beans are particularly susceptible during flowering where drought could cause flower and pod abortion. Additionally, SOURESHJANI et al., (2019) reported 60% yield losses of common bean due to flower and pod abortion caused by drought. Also, FAROOQ et al. (2017) reported that drought limits the productivity of grain legumes at all growth stages. It's more critical during reproductive and grain developmental stages which results in significant losses in grain yield. Therefore, it is crucial to improve common bean productivity to address global food and nutrition security challenges particularly in rural areas, where it served as source of protein.

Despite negative aspects of changes caused by drought, nitrogen metabolism (N) is one of the basic metabolic processes that affects the growth states, yield and stress tolerance in plants. Crops are capable of assimilating nitrate and NH<sub>4</sub><sup>+</sup>, thereby improving their nutrition while lowering their need on fertilizer (ELKOCA; TURAN; DONMEZ, 2010). There has been several reports on the association between proline accumulation and improvements in stress tolerance (HAGEMAN; VAN VOLKENBURGH 2021). ZHANG and LIU (2001), reported nitrogen application to wheat (*Triticum aestivum* L.) during grain filling enhanced the remobilization of stored carbohydrates from vegetative plant part to grain under moderate drought stress. Several studies have provided the evidence of the role of nitrogen in ameliorating the effects of drought stress by enhancing proline accumulation, glycine betaine

and soluble protein (MONREAL et al., 2007; SANEOKA et al., 2004; Zhang et al.,2007; ZHANG; LIU 2001)

Furthermore, according to IBRAHIM; IBRAHIM; ABD EL-GAWAD ( 2021), the physiology and biochemical function of folates in plants can be explained in a variety of ways. Folates are involved variety of amino acids production, such as methionine, glycine, tryptophan, glutamic acid, and valine, and may be involved in the biosynthesis pathways of many plant growth regulators, including auxins, polyamines, and ethylene(STAKHOVA; STAKHOV; LADYGIN, 2000). Folates are indirectly involved in the biosynthesis of porphyrins and S-adenosylmethionine (SAM), which results in the creation of chlorophylls and all isoprenoids, including carotenoids and -tocopherol (vitamin E), respectively (STAKHOVA; STAKHOV; LADYGIN, 2000). By triggering the glutathione-ascorbate ROS detoxifying cycle, folates can indirectly increase antioxidant capacity and induce stress tolerance mechanisms (GORELOVA et al., 2017).

More so, previous study reports that foliar application of nitrogen raised the relative water content (RWC) and nitrate reductase activity under short-term drought stress in maize (ZHANG et al.,2009). However, there is still a wide gap to be filled regarding the use of foliar fertilizer to improve tolerance to water deficit in common bean production. Therefore, this study aims to evaluate the effect of foliar application of organic fertilizer as N source in mitigating the negative consequences of water deficit on the growth and grain yields of common bean. in common bean (*Phaseolus vulgaris* L). We hypothesized that foliar application of organic fertilizer could enhance the drought tolerance of common bean through improvements in nitrogen metabolism.

## 2. LITERATURE REVIEW

Common bean production is compromised due to climate factors specially by salinity, temperature variations and water stresses, mainly drought, which limit the crop yield and yield quality, thereby leading to major socioeconomic and food insecurity (ZANDALINAS et al., 2021).

### 2.1 Economic Importance of Common Bean

The common bean is one of the most significant legumes in the world, as it contains high protein content, fiber and other critical minerals for humans. The common bean is one of the 727 genera and over 19,000 species that make up the Leguminosae family. This family includes several economically significant species, including,

**TABLE 1.** Leguminosae family names.

| SERIAL NO | SPECIE NAME | BOTANICAL NAME              |
|-----------|-------------|-----------------------------|
| 1         | Broad beans | <i>Vicia faba</i> L         |
| 2         | Peas        | <i>Pisum sativum</i>        |
| 3         | Chickpeas   | <i>Cicer arietinum</i>      |
| 4         | Lentils     | <i>Lens culinaris</i>       |
| 5         | Soy         | <i>Glycine max</i> L        |
| 6         | Common bean | <i>Phaseolus vulgaris</i> L |

Source: The Author.

The five *Phaseolus* species that are grown for human consumption worldwide (BITOCCHI et al., 2017; NADEEM et al., 2021).

**TABLE 2.** *Phaseolus* species names.

| SERIAL NO. | SPECIE NAME | BOTANICAL NAME                |
|------------|-------------|-------------------------------|
| 1          | Common bean | <i>P. vulgaris</i> L          |
| 2          | Runner bean | <i>P. coccineus</i> L         |
| 3          | Tepary bean | <i>P. acutifolius</i> A. Gray |
| 4          | Lima bean   | <i>P. lunatus</i> L           |
| 5          | Year bean   | <i>P. polyanthus</i> Greenman |

Source: The Author.

For both commercial and scholarly applications, *P. vulgaris* is the most often used species of bean (BITOCCHI et al., 2017; CORTÉS; BLAIR, 2018; ASSEFA et al., 2019). Due to its higher protein content (>22% of their dry weight) than some cereals like rice and wheat, common beans play a significant role in the human diet being considered as a staple food (EMANI; HALL, 2008; CASTRO-GUERRERO et al., 2016).

Common bean pods and seeds can be consumed in a variety of forms, including fresh, dry, canned and pickled. It is rich in protein, vitamins, complex carbohydrates, and minerals (Ca, Mg, K, Cu, Fe, Mg, and Zn) required for human healthy (MAROTTI et al., 2007; BLAIR, 2013; NADEEM et al., 2021). The common bean is one crop that helps smallholder farmers produce more crops, provides nourishment as a cheap source of protein, and generates cash as a high-value export crop (GETACHEW, 2019). Besides, common bean seeds are a good source of the lectin and amylase inhibitors (AI) needed to make insecticides (LEE; GEPTS; WHITAKER, 2002), as well as basic materials for creating compounds (PINTO; SANTOS; CHAMBEL, 2015). Compounds such as flavonoids, phenolic acid, coumestrol and galactooligosaccharides that may have protective effects against cancer (DÍAZ-BATALLA et al., 2006).

In addition to being a nutrition, as a leguminous, beans are a crucial because they improve soil structure, increase the amount of organic matter in the soil, accumulate nitrogen in soils due to its symbioses with specific bacteria, and use plant leftovers as a component of commercial feed combinations (BITOCCHI et al., 2017; YUVARAJ; PANDIYAN; GAYATHRI, 2020). Due to its high nitrogen content, common beans are utilized for a variety of purposes, including biological nitrogen fixation, livestock feed, and soil erosion control, (MUONI et al., 2019). Common bean and other legumes such as Peas (*Pisum sativum*), Lentils (*Lens culinaris*), and Chickpeas (*Cicer arietinum*) are also potential future alternatives in production of bioenergy and alcoholics (KARLSSON et al., 2015; LIENHARDT et al., 2019). The common bean can interact with Rhizobium bacteria to fix more than 160 kg of atmospheric nitrogen per hectare into the soil (BESHIR et al., 2015), which reduces the demand for nitrogen (N) fertilizers (PASTOR-BUEIS et al., 2021).

## 2.2 Detrimental Effect of Drought on Growth and Development of Common bean

The greatest obstacle to agricultural productivity is drought stress, considered as the major yield-limiting factor of crops that severely impairs plant growth, development, and yield. Making it challenging to sustain an agricultural system globally (MA; VOSÁTKA; FREITAS, 2019; AZEEM et al., 2022). Statistics from throughout the world showed that during the past 40 years, the number of regions impacted by drought has more than doubled, and losses to agricultural output from drought stress outweighed losses from all other abiotic stresses combined (FAO 2021). About 29% of the world's agricultural land was in cultivated drought-affected areas, measured as a percentage of all agricultural land (Statista, 2022).

A lack of water can inhibit the germination of seed in the early phases of plant growth, hinder cell elongation and consequently slow down plant growth (STEINER et al., 2017). Also, the development of floral initiation, pollination, seed development, seed yield, and seed quality are injured (ISLAM; HOQUE; HOSHAIN, 2022). Drought interrupts the mass movement of water-soluble nutrients, such as sulfate, silicon, nitrate, magnesium, and calcium, which are essential for normal plant development. Because less nitrate is absorbed from the soil during drought and important enzymes such as nitrate reductase (NR) may be affected (LI et al., 2020a). The activity of carbon and nitrogen assimilation enzymes such as glutamine synthetase (GS), glutamate dehydrogenase (GDH), sucrose synthase (SS), nitrate reductase (NR), glutamine synthetase (GS), and aminotransferase is also impacted by drought stress, changing the composition of carbon and nitrogen compounds in plants and reducing their resistance to drought stress (HUANG et al., 2018).

It influences several physiological processes in plants, such as cell wall turgidity, a slower rate of carbon absorption, and an increase in oxidative changes, which leads to a reduction in yield (CHOWDHURY et al., 2016; SREENIVASA et al., 2020). Due to drought stress, plant metabolism may result in the production of reactive oxygen species (ROS), which cause the oxidation of organic molecules such DNA, RNA, lipids, and proteins (ALI et al., 2014). In common bean, drought inhibits nitrogen fixation even earlier than photosynthesis. Besides limiting fixed nitrogen supply, drought also affects the absorption of mineral nutrients and translocation of assimilates, resulting in a drastic reduction in yield (ETIENNE et al., 2018).

Several studies revealed that common bean plant growth, seed yield, quality, and physiological and biochemical processes were severely hampered by drought stress (RASTI SANI et al., 2018). Farooq et al., (2017) stated that in all growing phases, drought reduces grain legume productivity. Its occurrence during the stages of grain development and

reproduction is crucial and typically causes large losses in grain yield. Furthermore, drought affects flowering times in plants (ALI et al., 2022)

Common bean are particularly vulnerable during flowering, a time when drought can significantly affect flower and pod abortion (GRAHAM; RANALLI, 1997), and cause yield loss of over 60% (SOURESHJANI et al., 2019). As reported by (HAGEMAN; VAN VOLKENBURGH, 2021), that drought caused both the wild and domesticated common beans lines to abort flowers and pod. Exposure to drought affects leaf area index, dry matter production, number of pods plant<sup>-1</sup>, number of seeds plant<sup>-1</sup>, hundred-seed weight, and seed yield (ASSEFA et al., 2017; KAZAI et al., 2019). Many physio-biochemical processes are disrupted by drought stress, which inhibits plant growth and development.

### **2.3 Effect of Drought Stress on Photosynthesis in Plant**

In photosynthesis, CO<sub>2</sub> and water within the chloroplast of plant cells produce sugars and O<sub>2</sub> as a by-product in the presence of light (DUURSMA; CHOAT, 2017). Therefore, Plants must capture light and use it during the photosynthesis process (ALLAKHVERDIEV, 2020). Plants are unable to absorb as photosynthetically active radiation (PAR) as they once could since they are shorter and have fewer leaves. This affects yield and photosynthesis (LI et al., 2020a).

The primary causes of photosynthesis slowing in common bean could be stomatal closure, because stomatal closure and mesophyll conductance, however, are the main factors restricting photosynthetic activity during plant adaptation to drought stress (FLEXAS et al., 2009; XU et al., 2019). As soils dries, Abscisic Acid (ABA) synthesis in above ground tissues increasingly occurs (BRUNETTI et al., 2019), leading to an increase in ABA in mesophyll cells that triggers stomatal closure (MCADAM; BRODRIBB, 2018). Numerous studies have demonstrated a negative correlation between stomatal conductance and the ABA content of leaves and xylem sap (BRUNETTI et al., 2019).

Because CO<sub>2</sub> is the substrate for plant photosynthesis, drought frequently lowers net photosynthetic rate ( $P_N$ ), which is accompanied by increased CO<sub>2</sub> diffusion resistance and decreased activity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in leaves (HAWORTH et al., 2019). LEITÃO et al., (2021), reported gas exchange parameters: assimilation rate ( $A$ ), transpiration rate ( $E$ ), stomatal CO<sub>2</sub> conductance ( $g_s$ ), substomatal CO<sub>2</sub>



concentration ( $C_i$ ) and relative water content of common bean decreased with the soil water content reduction to 40% of field capacity.

The relative content of chlorophyll, one of the primary chloroplast components, is strongly correlated with the photosynthetic rate (TALBI et al., 2020). Several studies have shown that drought stress can considerably reduce the amount of photosynthetic pigment (Chlorophyll a, Chlorophyll b, and Chlorophyll a + b) (DIKŠAITYTĖ et al., 2018). Under drought stress conditions, the decrease in chlorophyll content could be considered a symptom of oxidative stress as a result of pigment photo-oxidation and chlorophyll degradation (ANJUM et al., 2011; ASHRAF; HARRIS, 2013).

Drought induces oxidative stress by reducing the absorption of  $\text{CO}_2$ , which results in an excess of excitation energy and electron flow to  $\text{O}_2$ , leading to photo-oxidative stress and excessive generation of reactive oxygen species (ROS) over production (ZOU et al., 2009). Proteins, chlorophylls, membrane lipids, and nucleic acids are all damaged by ROS, which are extremely reactive species (HALLIWELL; GUTTERIDGE, 2015). (ASHRAF; HARRIS, 2013) have reported that drought and high temperature stress negatively affect the operation of both photosystems and hinder electron transport, causing a decrease in ATP and NADPH.

Plants have a variety of defense strategies to withstand environmental changes, such as drought (EL-SHESHTAWY et al., 2021). By the building up of metabolites such as sucrose, glycine betaine, polyamines, inorganic ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{H}^+$ ), amino acids (Pro), and amine compounds which takes place in plants to control osmotic potential during drought stress. These compounds often have low cellular toxicity, good solubility, and modest molecular weight. They can safeguard the protein activity and cell membrane structure, maintain the appropriate osmotic pressure level, and perform other similar functions (GHOLAMREZA et al., 2019; OZTURK et al., 2021), and activating the antioxidant defense mechanism to quickly remove ROS (GHONAIM; MOHAMED; OMRAN, 2021; ABU-SHAHBA et al., 2022). Non-enzymatic compounds like glutathione (GSH) and ascorbate (ASA) can also successfully scavenge ROS in addition to enzymatic antioxidant mechanisms. Therefore, drought has a negative impact on plant photosynthesis such reducing the impact of drought stress is very significant for preserving global food security.

## 2.4 Effect of Nitrogen on Photosynthesis

Plants grow better in soils that contain adequate levels of nutrients for growth (ZHAO et al., 2019). Due to the short cycle of common bean cultivation, the crop requires access to the soil's nutrients (GUIMARÃES et al., 2017). Nitrogen is the nutrient that the common bean plant absorbs the most, and it is crucial for the development of chlorophyll, vegetative growth, blooming, and the filling of pods and seeds. (TOSO et al., 2017). Nitrogen deficit inhibits plant growth and development, limits photosynthesis, reduces leaf area, induces plant senescence, and in the end, lowers plant production (CHEN et al., 2015; MU et al., 2016). The primary factor is that N, which is a crucial component of amino acids, proteins, cell walls, membranes, and nucleic acids, is therefore, essential for plant growth and development (MEHARG, 2012). Due to its high fixation capability when compared to other leguminous species, this element (N) can be provided to the soil by symbiotic nitrogen fixation (SNF) (PACHECO et al., 2020). Additionally, SNF process is extremely vulnerable to abiotic stress factors (drought, heat, soil acidity, low P, among others) that have an adverse effect on both plants and bacteria, reducing the uptake and utilization of N in marginal settings (POLANIA et al., 2016).

The estimated N requirement for crop growth is roughly 2.5 kg ha day<sup>-1</sup> during flowering and 3.5 kg ha day<sup>-1</sup> during grain filling (SORATTO; CARVALHO; ARF, 2004). However, insufficient N availability has a significant impact on photosynthesis's ability to absorb CO<sub>2</sub> (JIN et al., 2015).

An increase in N supply improves physiological processes like the photosynthetic rate, Since the amount of dry matter produced is entirely dependent on the plant's N status (SABERALI et al., 2016). An increase in N supply to the plant results in an increase in the plant's specific leaf area, the effectiveness of absorbing solar radiation, the rate of photosynthesis, and the levels of total chlorophyll (*Chl<sub>t</sub>*) and rubisco (SEEPAUL; GEORGE; WRIGHT, 2016). The relationship between leaf N concentration and photosynthesis has been reported in a significant body of literature ( BELOW, 2009; MU et al., 2016, 2018a).SUÁREZ et al., (2021), reported that increase in N supply in common bean resulted in specific leaf area (SLA), leaf nitrogen content and efficient photosynthetic use of N. Additionally, it increases the efficiency of the excitation energy that the photosystem II (PSII) open centers are able to capture (JIN et al., 2015). Additionally, N availability is closely related to leaf area and the leaves lifespan, both of which have a significant impact on biomass production. The amount of time that photosynthesis took place was determined by the stay green leaf, particularly for types with

fewer green leaves when they were mature. In order to increase agricultural productivity, it is crucial to understand the connection between N and photosynthesis.

However, a limited supply of N to the plant impairs the performance of photosystem II (PSII) and photosystem I (PSI), leading to a decline and degradation in protein content, particularly in the PSII reaction center, which lowers the rate of net photosynthesis (JIN et al., 2015; ABID et al., 2018). This correlated with the findings of ARAYA; NOGUCHI; TERASHIMA, (2010), which reported nitrogen limitation increases the carbohydrate content and reduces the photosynthetic activity of the leaves of common bean. Low N stress causes the chlorophyll content of plants like common bean, rice, soybeans, and populus, as well as plants like maize, grasses, and sorghum to drop (LUO et al., 2019; ZHONG et al., 2019). (LIMA; DA MATTA; MOSQUIM, 2000), reported that nitrogen deficiency in common bean causes chlorophyll contents declined. We could infer from those findings that various plants react differently to a Chlorophyll allocation to N stress. Chlorophyll fluorescence and the distribution of light energy are significantly influenced by N supply (HUANG et al., 2004; MU et al., 2017). In winter wheat, N deficit causes declines in maximum quantum efficiency of photosystem (Fv/Fm PSII), electron transport rate (ETR), and photochemical quenching (qP) (SHANGGUAN; SHAO; DYCKMANS, 2000; WANG; WANG; SHANGGUAN, 2016).

A number of environmental parameters, including light, CO<sub>2</sub> levels, temperature, water deficit and nutrient status, and other environmental conditions, have an impact on stomatal conductance. Stomatal conductance and/or movement are closely correlated with N. Nitrate, which serves as the primary source of N for plants, may control stomatal activity. As a NO<sub>3</sub><sup>-</sup>/H<sup>+</sup> exchanger, Chloride channel a (CLCa) is confined to the vacuole membrane and can selectively collect nitrate in the cell's main storage compartment (DE ANGELI et al., 2006). Stomatal opening is decreased in the nitrate transporter gene NRT1.1 mutant lacking the plasma membrane nitrate/H<sup>+</sup> co-transporter (GUO; YOUNG; CRAWFORD, 2003). Guard cells' SLAC1 (SLOW ANION CHANNEL-ASSOCIATED 1) and SLAH3 (SLOW ANION CHANNEL-ASSOCIATED 3) channels control slow type anion currents carried by the negative charge of chloride (GEIGER et al., 2011). Nitrate presence promotes the opening of major anion channel SLAC1 and thus drives stomatal closure. By controlling turgor and hormones, stomatal conductance increases when N supply rises (HILLERDAL-HAGSTRÖMER; MATTSON-DJOS; HELLKVIST, 1982; MAKINO et al., 1997; BROADLEY et al., 2001). In addition, hydrogen peroxide H<sub>2</sub>O<sub>2</sub> builds up in conditions of N deprivation and causes stomata to close (TEWARI; KUMAR; SHARMA, 2007). The pattern resembles the correlation between *g<sub>s</sub>* and leaf N content.

The ratio of photosynthetic rate to leaf nitrogen, or PNUE, is a key indicator of a plant's photosynthetic properties (ZHONG et al., 2019). It is a significant marker of the plant's ability to utilize nutrients in its leaves and its physiological traits, which accurately reflect the allocation of nitrogen and the plant's overall photosynthesis (ZHONG et al., 2019; NASAR et al., 2021). The relationship between the leaf's photosynthetic properties and photosynthetic nitrogen directly affects the plant's PNUE (HIKOSAKA, 2004; ZHANG et al., 2017). SUÁREZ et al., (2021), reported that the improved photosynthetic N use efficiency (PNUE) and the capacity to partition photosynthates to grain are primarily responsible for the common bean's response to N supply, which is also genotype-dependent. The plant's PNUE and leaf nitrogen usage rate increase as photosynthetic rate increases (NASAR et al., 2022). As a result, understanding photosynthesis and plant PNUE is crucial to understanding how crops are affected.

## **2.5 Impact of Nitrogen Metabolism on Drought Stress**

Since nitrogen (N) is a component of amino acids, the building blocks of proteins and enzymes, nitrogen (N) is necessary for all general plant processes. In addition, amino acids serve as precursors or N donors for a variety of essential substances, such as nucleic acids, hormones, chlorophyll, ureides, and other metabolites needed for both basic metabolism and more complex biological processes (ZRENNER et al., 2006; BITTSANSZKY et al., 2016; ZÜST; AGRAWAL, 2016). Additionally, the primary long-distance forms of N in plants are amino acids (THOMPSON; VAN BEL, 2012; TEGEDER; HAMMES, 2018). While proteins, peptides, and amino acids can be absorbed from the soil (CHAPIN et al;1993; NÄSHOLM; KIELLAND; GANETEG, 2009; TEGEDER; RENTSCH, 2010), ammonium and nitrate are frequently preferred (CRAW VON WIRÉN, 2004; KRAPP et al., 2014). It is widely acknowledged that plant absorbs N through roots in the form of  $\text{NO}_3^-$  and transports to the leaves for N assimilation. Nitrate reductase in the cytoplasm converts  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , and  $\text{NO}_2^-$  is delivered to the chloroplast and then converted into  $\text{NH}_4^+$  by nitrite reductase. (XU; ZHOU, 2006), reported that  $\text{NH}_4^+$  is converted to glutamate and glutamine through the glutamine synthetase (GS), glutamate synthetase (GOGAT). The accumulation of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  in the nitrogen metabolism process is essential for maintaining the activities of key enzymes, such as GS and GOGAT for plant growth and development (NGUYEN et al., 2005). Important metabolic indicators of drought tolerance are thought to be the activity of GS and GOGAT (NAGY et al., 2013; SINGH; GHOSH, 2013).

The key regulatory step in the N assimilation process is catalyzed by nitrate reductase (NR), and the availability of nitrate, light, and water strongly influence this enzyme's activity (CHAUVIN; MEYER, 2002; ZHANG et al., 2014; CHAMIZO-AMPUDIA et al., 2017). Additionally, amino acids (such as glutamine) present signals governing absorption and assimilation by impacting both the activity of nitrate transporters and the activity of nitrate oxidases (VIDMAR et al., 2000; THORNTON, 2004; MILLER et al., 2008) and NR (DZUIBANY et al., 1998; MIGGE et al., 2000; FAN et al., 2006). Increased N uptake has frequently been caused by overexpression of nitrate reductase (NR) or nitrite reductase (NiR)

Because of their ability to synthesize and store a variety of organic solutes in their cytoplasm, plant can withstand droughts (MWENYE et al., 2016; RAO; CHAITANYA, 2016). Another significant byproduct of the metabolism of nitrogen is proline. Proline is one of these organic solutes which is crucial in protecting cells from harm brought on by drought stress (ANDRADE et al., 2016; RAO; CHAITANYA, 2016). Few findings reported that under drought stress condition nitrogen assimilation products, including  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and proline can regulate the selective absorption and transport of ions in plants and maintain the balance of physiological metabolism. Dipp et al., (2017), reported increased level of proline in common bean root under intermittent drought stress during reproductive stage that made it possible for osmotic adjustment and the maintenance of a middle-ground stomata closure throughout the day, keeping the intrinsic water use efficiency (WUE) steady in drought stress conditions.

Similarly, levels of amino acids rise, has been reported and it also has been seen that organic acids like malate, fumarate, and citrate also rise under drought stress. These compounds may also function to protect subcellular structures and macromolecules against the harmful effects of drought stress (MULLER et al., 2011). Additionally, Andrade et al., (2016) reported doubling of amino acids levels in common bean during pod filling stage under drought stress condition when compared with irrigated one.

## **2.6 Effects of Fertilizers Application in Improving Tolerance to Drought Stress**

The foliar application might be regarded as one of the most popular techniques, which is used to provide the essential nutrients to plants in sufficient quantities, enhance their nutritional status, and raise crop output and quality (SMOLEŇ, 2012). The application of amino acid fertilizers can reduce the impact of external abiotic stress factors such water stress, enhance soil water-holding capacity and root conformation, and stimulate root growth with favorable

impacts on nutrient and water use efficiency and production. Consequently, the usage of amino acid fertilizers in agriculture has increased dramatically during the past ten years (BULGARI et al., 2015; VAN OOSTEN et al., 2017). Foliar applications could also be employed for a variety of objectives, including as reducing the negative effects of various conditions (such as heat, drought, cold, etc.) and spraying various plant nutritional substances (such as basic amino acids, peptide chains, growth regulators and stimulators, sugars, disaccharides), insecticides, and nanomaterials (EL-RAMADY, 2014; DE SOUZA SIMÕES et al., 2017). Plant amino acid fertilizers also include a variety of bioactive compounds that can enhance physiological processes that promote plant development and increase nutrient usage efficiency without negatively affecting crop output and the quality of the finished product, while also reducing the inputs of chemical fertilizers (BULGARI et al., 2015; KOLEŠKA et al., 2017).

The impact of amino acid fertilizers, however, may vary from species to species and is heavily influenced by environmental conditions both before and after administration as well as the dose and timing of treatment (KUNICKI et al., 2010; ROUPHAEL et al., 2015). Vegetable crops such as the common bean are drought-sensitive, and crop productivity may suffer from water stress (SOURESHJANI et al., 2019). As well as the chemical content of seeds and pods (HUMMEL et al., 2018). The use of amino acid fertilizers on common bean plants has received relatively little attention in the literature, but other legume species, particularly those that are drought-stressed, have been the subject of several studies testing their effects. By using amino acids as carbon and nitrogen pools, foliar amino acid treatment on faba beans (*Vicia faba* L.) subjected to salt stress demonstrated considerable ameliorative benefits that further boosted photosynthetic apparatus efficiency (SADAK; ABDELHAMID; SCHMIDHALTER, 2015). In another study, (DOURADO NETO et al., 2014), suggested that using hormones with fertilizer action (a mixture of kinetin, indole butyric acid, and gibberellic acid) on common bean plants during seed treatment, planting, or foliar spraying may improve the number of grains per pod and grain output. Abu-muriefah, (2013), indicated increased growth and yield of common bean under drought stress using foliar application of Chitosan fertilizers and the beneficial effects of aqueous extracts of garlic and moringa leaves on the yield and chemical makeup of snap beans (ELZAAWELY et al., 2018).

However, most of the studies regarding the mitigating effects of foliar application of nitrogen fertilizers to abiotic stressors like drought refer to other crops. According to Petropoulos et al., (2020), unlike other plant nutrients, nitrogen (N) is needed by plants in relative high concentration. Numerous studies have demonstrated how nitrogen can reduce the consequences of drought stress by promoting proline accumulation, glycine betaine and soluble

protein (MONREAL et al., 2007; ZHANG et al., 2007). Proline accumulation has been discovered to serve adaptive roles in stress tolerance (YOU et al., 2019). According to Zhang et al., (2009), under short-term drought stress, foliar nitrogen treatment increased the relative water content (RWC) and the nitrate reductase activity in maize. Conclusively, Nitrogen application could boost plants' resistance to drought in addition to providing a nutrient for plant growth, which would increase yield when drought stress is present (CHIPMAN; RAPER JR; PATTERSON, 2001; LI et al., 2009).

### **3.GENERAL OBJECTIVE**

The main objective of this work was to evaluate the effect of foliar application of amino acid enriched fertilizer as N source in mitigating the negative consequences of water deficit on the growth and grain yields of Common bean.

#### **3.1 Specific objectives**

The specific objective was to:

- Determine the efficiency of the proposed amino acid enriched fertilizer in enhancing N uptake for improving the crop qualitative and quantitative parameters.
- Investigate the role of foliar application of amino acid enriched fertilizer in improving accumulation of amino acid and proline, which enhance tolerance to water stress.

#### **3.2 Hypothesis**

We hypothesized that foliar application of amino acid enriched fertilizer as N source to Common bean could enhance tolerance to water deficit and improve grain yields in crop through the improvement of N metabolism in these plants.

## **4. METHODOLOGY**

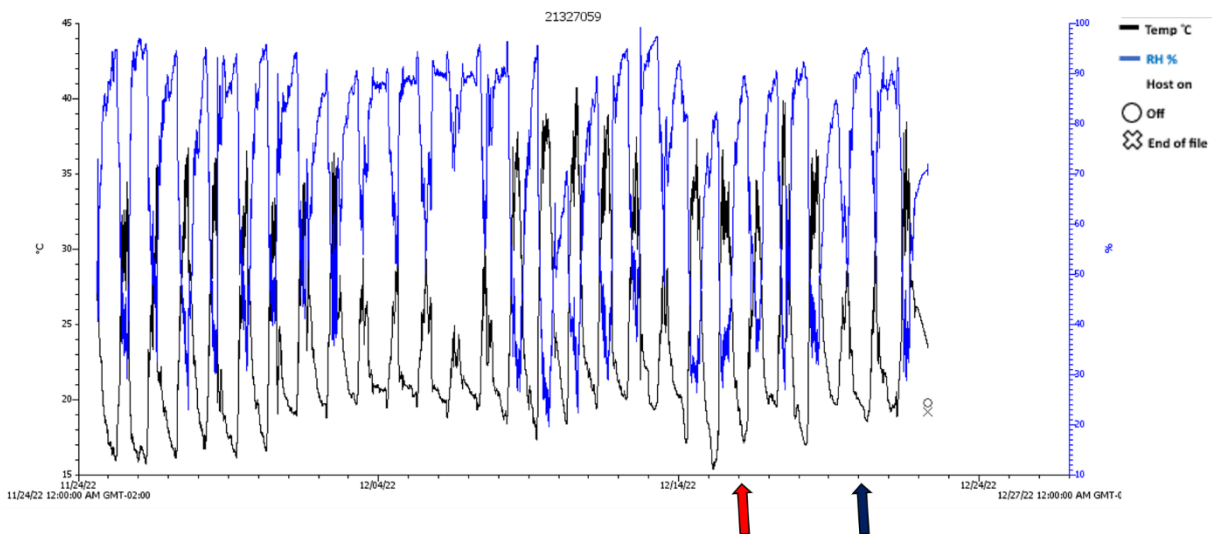
### **4.1 Study Site Description and Climatic Variables**

The experiment was conducted in a greenhouse of the Plant Physiology Sector of the Federal University of Lavras, Minas Gerais, Brazil, located between longitudes 44°59'24.30"

and 44 57°36'58" W and latitudes 21°14'28.93" and 21°13'10.91" S. The climate of the region is Cwa by the Köppen classification having an average annual temperature of 20.4 °C and an average annual rainfall of 1460 mm.

The greenhouse's climatic conditions during maximum stress and rehydration conditions in December 2022, while plant development and physiological measures were being taken, were as follows (Fig. 1): Using a Datalogger model HT- 500 (Instrutherm), measurements taken every 30 minutes yielded relative humidity (RH) values of 90% and air temperature (To air) values of 35°C, and 28°C temperature (To air), 95% relative humidity (RH) values respectively.

**Figure 1.** Schematic representation of climatic variables under maximum stress and rehydration conditions



Source: The Author.

## 4.2 Treatment and Experimental Materials

The experimental treatments are composed of two (2) factors arranged in a factorial combination (2x2) in a completely randomized design (CRD). The experiment consists of four treatments, T1-T4 with five (5) replications. The factors are: i. water availability – well watered and water withholding; ii. Foliar fertilization – with foliar fertilization and without foliar fertilization (Table 3).



**Table 3- Treatments description**

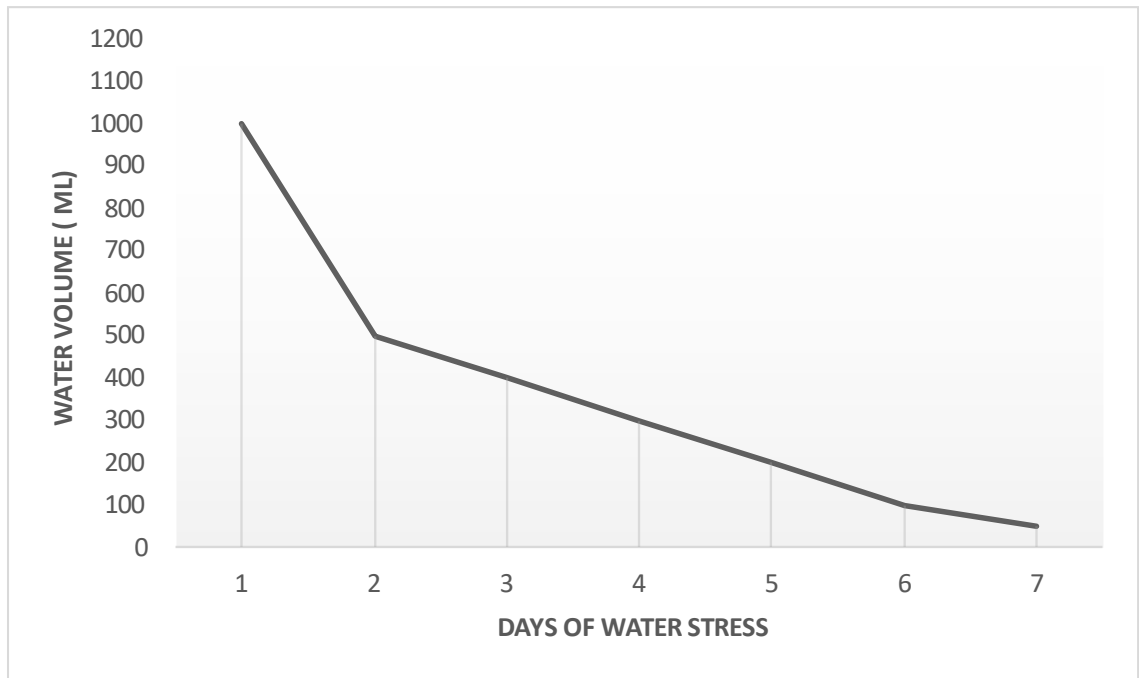
| Treatments | Description                 |
|------------|-----------------------------|
| <b>T1</b>  | WW + MOCK                   |
| <b>T2</b>  | WD + MOCK                   |
| <b>T3</b>  | WW + 0.4 L ha <sup>-1</sup> |
| <b>T4</b>  | WD + 0.4 L ha <sup>-1</sup> |

WW: well-watered; WD: water déficit

### 4.3 Water Deficit Application and Rehydration

The water withholding was initiated at V4 stage, when the plants attained first three trifoliolate leaves. Prior to the application of stress. The plant pots were irrigated with 1000 ml of water for the soil to attained saturation point on day three after the first application of the product. Twenty-four hours later, ten pots of the controlled plants were weighed using scale, and the values summed and divided by the number of the control plants weighed in order to determine the average of water loss in a daily basis. The same control plant pots were weighed again after twenty-four hours using same balancer when the soil reached field capacity. The second day total values of the weighted control plants vases were subtracted from the first day total values. The 100% difference of the first day and second day total values of water were applied in milliliter (ml) to all irrigated plants and 50% to all water stress plants until stress was noticed on the water deficit treatments.

**Figure 2.** Schematic representation of water deficit application to stress plants.

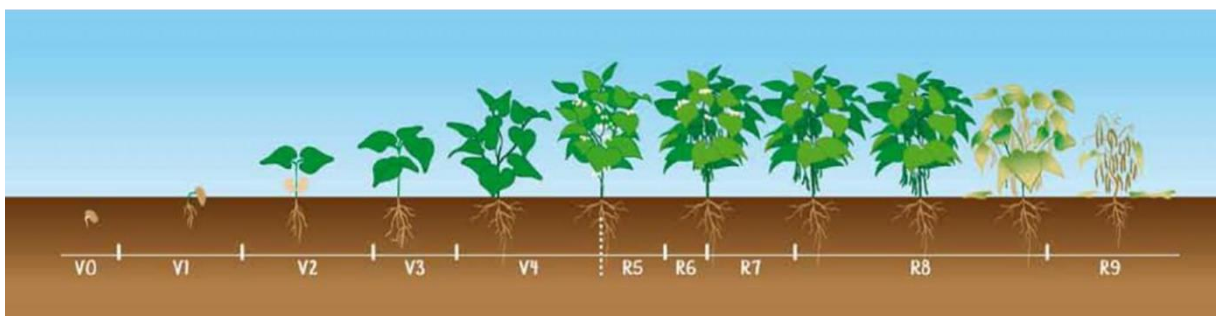


Source: the author.

#### 4.4 Fertilizer Foliar Application

An amino acid enriched fertilizer (Vislumbre®; AgroPrime, Brazil) were applied on the leaves of the common bean at V4 stage (pre-Anthesis stage) on the treatments 3 and 4 that required Vislumbre® application, when the plants present first three trifoliolate leaves in a dose equivalent to  $0.4 \text{ L ha}^{-1}$ , (96  $\mu\text{L}$  mixed in 36 ml of water) and applied to sixty (60) plants using portable hand sprayer. The second application of the same quantity of product was applied at R5 stage seven days after the first application on the same number of plants. This was to improve resistance to water deficit stress on the flower abortion.

**Figure 3.** Phenological phases of common bean plants



Schematic representation of phenological phases of common bean plants. The Vislumbre® applications were done in V4 and R5 stages. Source

Source :Embrapa, 2018

#### 4.5 Fertilizer and Variety Description

Vislumbrepod® is mineral fertilizer that has characteristics of stimulating plant metabolism and activate routes aimed at preventing and protecting against biotic and abiotic stressors as well as antioxidant properties. Vislumbre is a foliar mixed mineral fertilizer containing 5% Nitrogen and 5% Phosphorus solution, sourced from phosphoric acid and urea. It also includes complemented amino acids at 18.45%. The product enhances plant growth and development by providing essential nutrients and amino acids, leading to improved nutrient efficiency and stress tolerance. It is manufactured by Prime Agro products Agricultural limited, Porto Alegre, Brazil.

The common bean variety was BRSMG MARTE, which is characterized by resistant to diseases, high yield potential and early maturing with 75-83 days developmental cycle in addition to its acceptance by both producers and consumers (SEAPA, 2023).

#### 4.6 Control of Pests and Diseases

The experiment was maintained under optimum weather conditions and under pest and disease control. The weeds were manually controlled using hand-picking periodically. In addition to this, regular inspection was performed to checked the health status of the plants. The fungicide FORTH FUNGICIDA Difenconazole (30 ml) from Forth Jardim was applied at right dose against fungal infection.

#### 4.7 Gas Exchange and Chlorophyll contents Measurements

The Gas exchange and Chlorophyll contents measurements was collected two (2) times, during the stress and under rehydration conditions. Data on chlorophyll *a* and chlorophyll *b* were collected using electronic chlorophyll content meter clorofiLOG (model CFL1030, Falker, Brazil), from the central portion of the fully expanded leaf. The gas exchange measurements were performed on stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), net photosynthesis ( $A$ ), water use efficiency ( $WUE$ ), leaf temperature ( $T_{leaf}$ ), intrinsic water use efficiency ( $A/g_s$ ), water vapour pressure deficit at the leaf ( $V_{pdl}$ ), air temperature ( $T_{air}$ ). The measurements were done on the youngest fully expanded leaves using infrared gas analyzer (IRGA) model LI-6400 (LICOR inc., USA) at 8 a.m. until 12 a.m. This was to determine the photosynthetic ability of

the variety within a specific level of growing condition. It was used  $PAR = 1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $C_i = 400 \mu\text{mol mol}^{-1}$ .

#### 4.8 Conduct of the Experiment

The experiment was conducted with 10 kg red–yellow Argisol soil in combination with loam soil (EMBRAPA, 2016) in 2: 1 ratio, contained in a 5 L plastic pots. In each pot, five seeds were sown, 3-6 cm depth and later thin to two plants per pot when the common bean plants attained phenological stage V3 i.e., around twelve days after sowing (DAS).

Prior to commencement of the experiment, 1 ½ kg of NPK 4:14:8 were applied during soil preparation. Ten days after sowing, 2 ml of micronutrients mixed in 100 ml of water and later 2 ml solution was applied to each pot immediately after thinning. This was because plants need it in small quantity and also to reduce competition among the plants. Additionally, 20 g of ammonium sulphate was applied at V3 stage per pot.

A day after the second application of the Vislumbre®, the first data on morphology, gas exchange and biochemical analysis were collected and thereafter irrigation resume for water deficit treatments for five days after which second data on the same parameters were collected. Production data on number of pods, grains number, seed mass per grain and seed weight were collected when the crop complete its life cycle.

On the R6 stage, one week after the second application of the product during the water deficit condition, data on water potential was collected around 4am before the sun rise using pressure chamber model 3005 (Soil Moisture Equipment Corp. Santa Barbara CA, USA) on the center trifoliolate leaves of the plant' to assess the water stress level. The same method was repeated five days after rehydration of the plants.

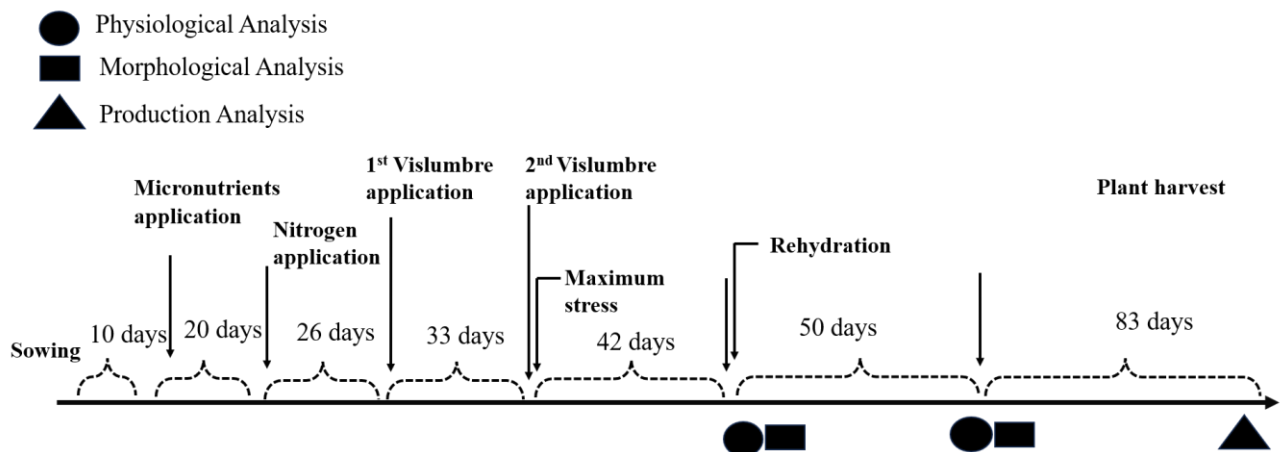
Similarly, at the same day of water potential data collection, morphological data on leaf area (Mature and Immature leaves) were collected using (Easy Leaf Area) an automated digital image analysis for rapid and accurate measurement of leaf area (EASLON; BLOOM, 2014) application downloaded from play store. Data on plant height was also collected using tape. So also, data on number of flowers, number of leaves and number of nodes were collected to determine the water stress level of the plants. Data on relative water content (RWC) was collected according to (BARR; WEATHERLEY, 1962). One youngest fully expanded leaf. Sample was cut from trifoliolate leaves, put in a plastic bag, and then put in a cooler container containing ice, and taken to the laboratory for the measurement. This was to reduce

transpiration. Twenty leaves discs were collected (10cm) from the leaf sample to determine fresh weight thereafter put it in petri dish and hydrated with non-ionized water for twenty-four (24) hours. After hydration, the samples were taken out of water and dry of any surface moisture quickly and lightly with tissue paper and immediately weighed to obtained turgid weight (TW). Samples were then oven dried at 65 °C for twenty-four (24) hours and weighed to determine dry weight (DW). The relative water content (RWC) was calculated using the equation:  $RWC = \left[ \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \right] \times 100$ , according to the methodology of (BARR; WEATHERLEY, 1962).

At R6 stage, two young fully expanded trifoliolate leaves were collected and put in a plastic bag covered with aluminum foil contained in a cooler container containing liquid nitrogen and later stored in -86 °C refrigerator to determine enzyme activity. Another two trifoliolate leaves were collected from each pot of the experimental treatments and dried in a forced air oven at 65 °C until constant weight was obtained for the determination of macro and micro molecules. All the above-mentioned parameters were collected at R7 stage five (5) days after the plants were rehydrated.

Finally, at R9 stage, production data on number of pods per plants were collected after which the plants were harvested. The pods were threshed and data on grains number per plant, seed weight and seed mass were also collected. This was simply to determine whether the Vislumbre® has improve tolerance to water deficit thereby resulted in increased yield and seed quality of the common bean under water deficit condition.

**Figure 4.** Schematic flowchart of the study



The arrows indicate application of different activities, while the cycle, Rectangle and Triangle indicates physiological, morphological and production analysis respectively. The days interval were also shown.

Source: the author.

#### 4.9 Lipid Peroxidation and Hydrogen peroxide analysis

Quantification of lipid peroxidation was conducted biochemically in accordance with (BUEGE; AUST, 1978). 400 mg of fresh matter from fully expanded leaves was collected and grinded in liquid nitrogen and homogenized in 1500  $\mu$ L of trichloroacetic acid. The reaction was stopped by rapid cooling in ice and read in a spectrophotometer at 535 nm and the peroxidation was expressed in nmol of MDA.g<sup>-1</sup>FM.

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels were quantified by the method of (VELIKOVA; YORDANOV; EDREVA, 2000) 0.1 g of leaves was used and macerated in liquid nitrogen and homogenized with 1500  $\mu$ L of 0.1% Trichloroacetic Acid (TCA). The samples were centrifuged and the reaction was performed with 10 mM potassium phosphate buffer (KH<sub>2</sub>PO<sub>4</sub>), pH 7.0 and 1 M potassium iodide (KI). The samples were analyzed in a spectrophotometer with absorbance reading at 390 nm and the levels of H<sub>2</sub>O<sub>2</sub> were quantified using a standard curve.

#### 4.10 Extraction and Quantification of Proline

The extraction of proline was determined using (BATES; WALDREN; TEARE, 1973). In which 100 mg of the dry plant materials (leaves sample) was homogenized and macerated with 5 mL of 3% sulfosalicylic acid and placed in 20 mL falcon tubes. Later, the extracted material was shaken at room temperature for 60 minutes and then centrifuged at 5,000 rpm for 10 minutes to collect the supernatant. The proline concentration was determined in test tubes by reaction with the sample, ninhydrin reagent (2.5 g of ninhydrin, 60 mL of acetic acid and 40 mL of 6 M phosphoric acid), water and acetic acid for 60 minutes at 100 °C. The reaction was stopped in an ice bath. The concentration of proline was determined using a standard curve and read at 520 nm in a spectrophotometer.

#### 4.11 Antioxidant Enzymatic System Analysis

The enzymatic antioxidant system activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were quantified, in which the enzymatic extraction was performed according to (BIEMELT et al., 1998). 200 mg of fresh matter (leaves sample) were macerated in liquid nitrogen with 50% PVPP (Polyvinylpyrrolidone) added to 1.5 mL of extraction buffer composed of 400 mM potassium phosphate (pH 7.8), 10 mM EDTA, and 200 mM ascorbic acid. The solution was then centrifuged at 13,000g for 10 minutes at 4 °C and the supernatant collected to quantify enzyme activities. SOD activity determination was based on

the enzyme capacity of inhibiting the photoreduction of nitro blue tetrazolium (NBT) (GIANNOPOLITIS; RIES, 1977). Absorbance readings was made at 560 nm. A unity of SOD corresponds to the number of enzymes able to inhibit 50% of NBT photoreduction in the assay conditions.

HAVIR AND MCHALE (1987) findings were used to determine the CAT activity. Based on the hydrogen peroxide consumption and the decrease in absorbance at 240 nm, every 15 seconds for 3 minutes, the enzyme activity was estimated. We assumed a molar extinction coefficient of  $36 \text{ mM}^{-1} \text{ cm}^{-1}$  (AZEVEDO et al., 1998). APX activity was examined according to (NAKANO; ASADA, 1981) APX using ascorbate oxidation at 290 nm and a molar extinction coefficient of  $2.8 \text{ mM}^{-1} \text{ cm}$ .

#### **4.12 Estimation of Carbohydrates and Proteins Analysis**

The method of (ZANANDREA et al., 2010) for the analysis of total soluble sugars, reducing sugars, amino acids and proteins. 200 g of dry matter (DM) was used in 10 mL of 0.1 M potassium phosphate buffer pH 7.0, followed by putting it in water bath for 30 minutes at 40 °C. Afterwards, the sample was centrifuged at 5,000 rpm for 10 minutes. Thereafter, it was stored in properly identified plastic jars at -20°C. The Aliquots of the supernatant were used for the analysis of the above-mentioned carbohydrates and protein. After the extraction, the pellet was resuspended with 4 mL of 200 mM potassium acetate buffer pH 4.8. The samples were thawed in a water bath at 40°C. It was added 1 mL of the 1 mg/mL amyloglucosidase enzyme solution (1 mg of the enzyme in 1 mL of 200 mM potassium acetate buffer pH 4.8). It was incubated in a water bath at 40 °C for 2 hours, and centrifuged at 5,000 rpm for 10 minutes. The supernatant was collected and stored in properly identified tubes at -20 °C.

Starch and total soluble carbohydrates were quantified by the anthrone method enzyme according to (YEMM; WILLIS, 1954). The sample was added first and then the anthrone reagent. The tubes were shaken and placed in a water bath at 100 °C for 3 minutes. This process, they were cooled at room temperature or on ice and read at 620 nm in a spectrophotometer. In leaf samples, total protein (mmol/g) was determined by the method of (LOWRY, 1951).

#### 4.13 Nitrogen total Analysis

The total nitrogen was determined using (KJELDAHL, 1883) method, where 100 mg of dry leaves (DM) samples were collected and the digestion flask on assembly was placed. The digestion flask was filled with concentrated sulfuric acid of 12 -15 ml. 7 mg of copper and potassium sulfate was added. The solution was kept at 370-400 °C temperature in the digestion flask which lasted for 60-90 minutes and finally the flask was cooled by adding 250 ml of water. The ammonia was separated by distillation method and quantify by titration method.

#### 4.14 Extraction and quantification of Nitrate

Nitrate was extracted and quantified according to (CATALDO et al., 1975) method in which 100 mg of plant dry matter (DM) was used in 10ml of deionized water in falcon tubes. The samples were incubated in a water bath for 1 hour at 45°C. Followed by samples centrifuged at 5000g for 15 minutes after which the supernatant was collect in 20ml falcon tubes.

The analysis was performed by mixing SA- H<sub>2</sub>SO<sub>4</sub> – 5% salicylic acid in concentrated sulfuric acid 5g, salicylic acid in 100ml concentrated H<sub>2</sub>SO<sub>4</sub> and also slowly added NaOH 2M in 100 ml of water. Followed by addition of 100µg/ml of NO<sub>3</sub><sup>-</sup> solution. After addition of NaOH the process was allowed to cool down at room temperature and read the absorbance at 410 nm in a spectrophometer. The value was then expressed in µg NO<sub>3</sub><sup>-</sup>/g MS.

#### 4.15 Extraction and quantification of Ammonium

Using the (MCCULLOUGH, 1967) methodology, extraction, and quantification were carried out. 60 mg of fresh matter (Fm) was macerated in liquid nitrogen. In Eppendorf, 1500µl of ultra-pure water was added after that, the samples were then centrifuged at 4°C for 20 minutes at a speed of 4000 rpm after being incubated at 80°C in a water bath for 10 minutes. In a 2 ml Eppendorf, the supernatants were then collected.

The ammonium concentration was determined by preparing two solutions as reaction media viz: solution 1 consisted of 48.29 mM phenol and 0.077 sodium nitroprusside. While solution 2 comprised of 56.81 mM NaOH, 0.068 M Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O and NaOCL.

After which, 100 µl of water-soluble extract was pipetted in an Eppendorf, then 500 µl were added of both solution 1 and 2 and incubated for 60 minutes in water bath. The reaction



was stopped in an ice bath. The ammonium was determined from a standard curve and absorbance was read at 630 nm in a spectrophotometer.

#### **4.16 Statical Analysis**

The data collected was subjected to Analysis of variance (ANOVA), using the statistical software R (R i386 3.3.3). Where the significant difference exists between the treatments, means were compared using Scott-Knott test at 5% probability of error ( $\leq 0.05$ ). The software GraphPad Prism 5.0 (Graphpad Inc.) was used to create the charts.

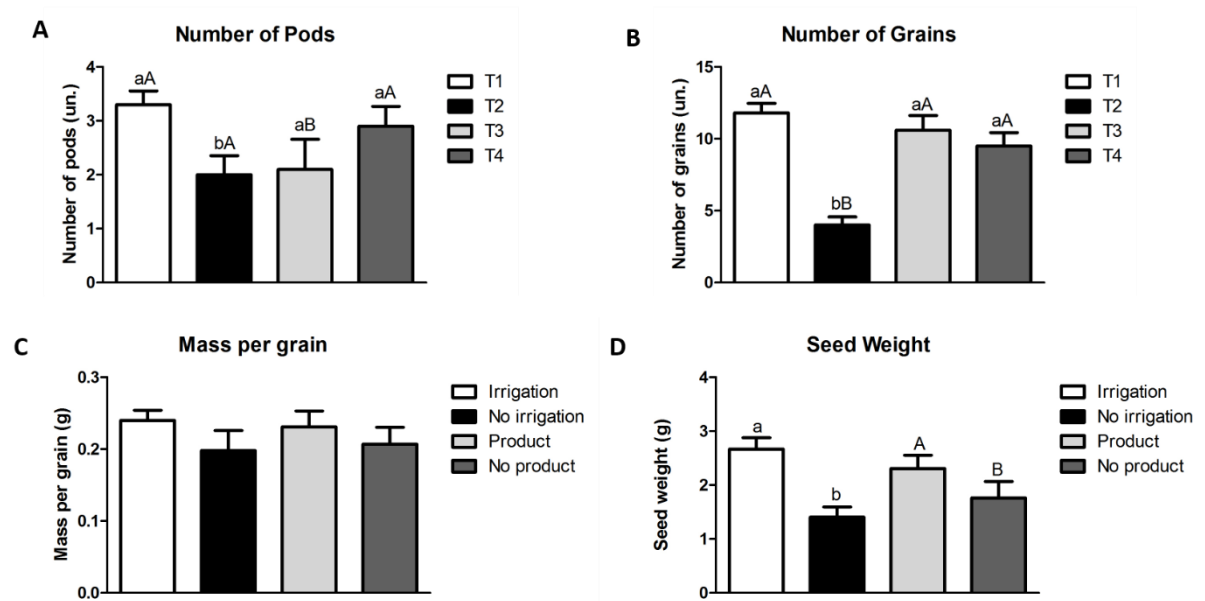
### **5. RESULT**

#### **5.1 Effects of fertilizer on Number of pods, Number of grains, Mass per grains and Seed weight, in Common Bean Plants under Maximum Stress and Rehydration conditions**

Regarding number of pods, the pods were significantly higher in T1 compared to T3. Similarly, increased pods number was observed in T4 compared to T2 (Fig.4A). Additionally, fertilizer caused increased in grains number when compared T4 to T2, while no statistical difference was observed between T1 and T3 (Fig.4B).

Moreover, no statistical difference was observed in all the treatments with regards to mass per grain. (Fig.4C). Finally, seed weight showed no statistical difference between T4 and T2, although, T4 had higher value than T2. Similarly, no statistical difference was observed when compared T1 to T3, however, T3 had shown higher value than T1 (Fig.4D).

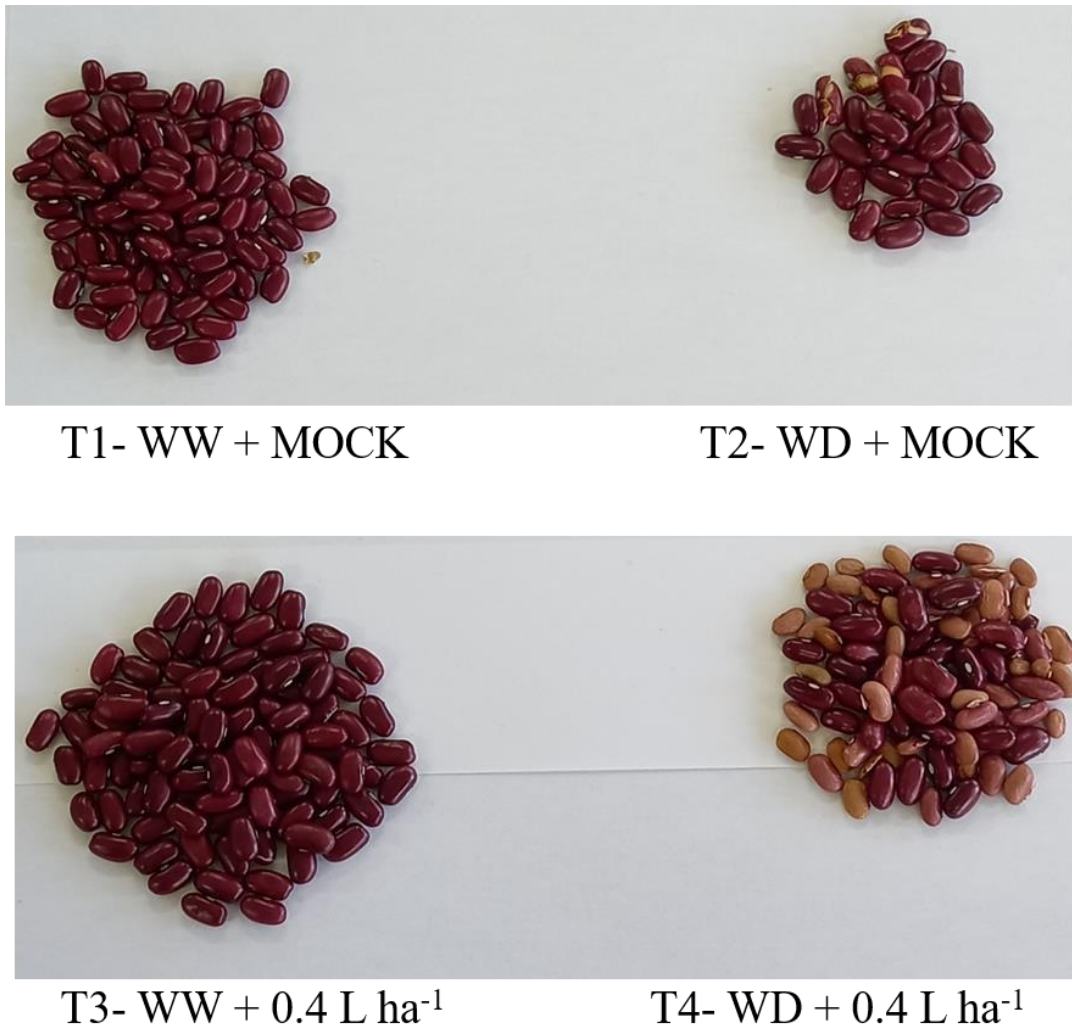
**Figure 5.** Yield data of number of pods



Yield data of number of pods (A), number of grains (B), mass per grains (C) seed weight (D) under maximum stress and rehydration conditions, and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5\pm se$ ). T1-WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

Source: the author

**Figure 6.** Production grains yield of the treatments.



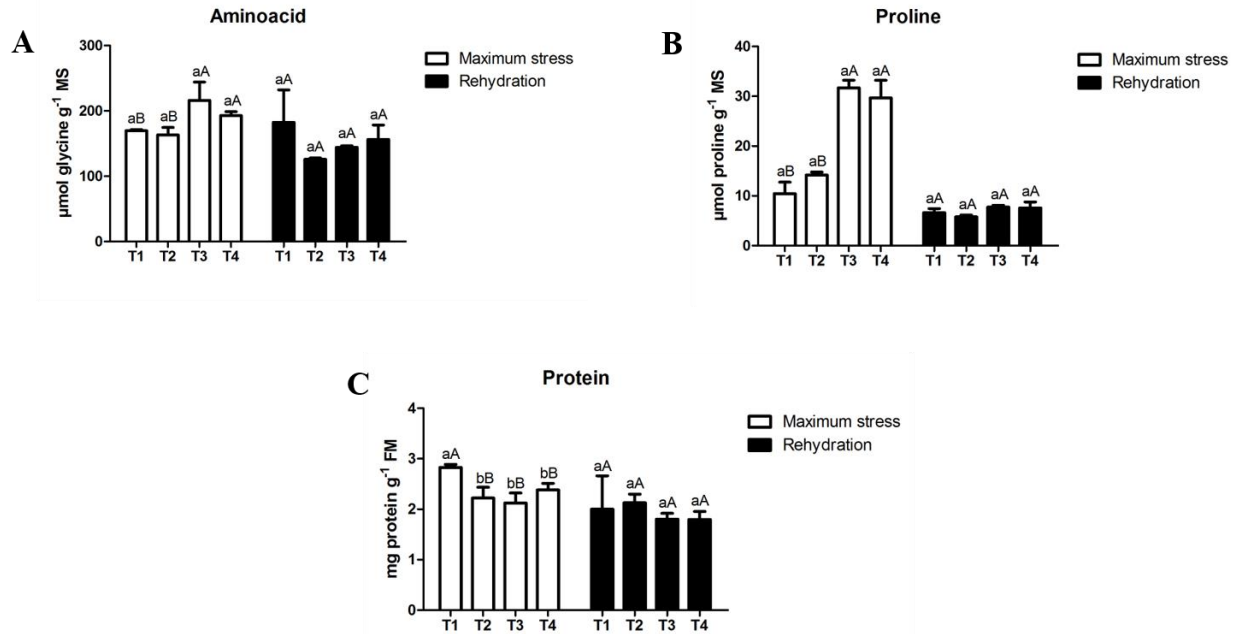
Source: the author

### **5.2 Effects of fertilizer on Amino Acids, Proline, And Proteins in Common Bean Plants under Maximum Stress and Rehydration**

During maximum stress, T3 presented a higher concentration of amino acids when compared with T1, and also T4 showed a significantly higher concentration of amino acids compared with T2. However, no statistical difference was observed in the rehydration period (Fig.6A). Vislumbre® caused increased proline concentration in leaves in T3 compared with T1. Similarly, T4 showed significantly higher values compared with T2 under maximum stress conditions. But with the return of irrigation, all the treatments were statistically equal (Fig.6B).

Protein concentration was elevated significantly in T1, compared to T3. There was no statistical difference between T2 and T4 in the period of water stress. However, all the treatments were statistically the same during rehydration (Fig.6C).

**Figure 7.** The concentration of amino acids , proline, and protein



**Figure 7.** The concentration of amino acids (A), proline (B), and protein (C) under maximum stress and rehydration conditions in common bean plants and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5 \pm se$ ). T1- WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

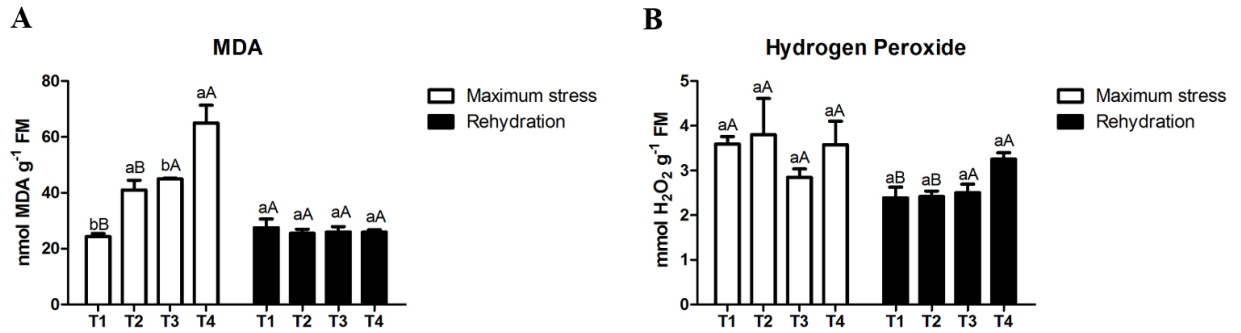
Source: the author

### 5.3 Effects of Vislumbre® on Lipid Peroxidation (MDA) and Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) Production on Common Bean Plants under Maximum Stress and Rehydration

#### Conditions

Under maximum stress T4 showed a higher lipid peroxidation compared to T2. Similarly, (T3) also presented more concentration of (MDA) when compared with T1. While in the rehydration condition, no statistical difference was observed among the treatments (Fig. 7A).

The production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was higher in all the treatments under maximum water stress conditions. In the rehydration period, a higher H<sub>2</sub>O<sub>2</sub> production was observed in T3 compared with T1 and also T4 when compared with T2 (Fig.7B)

**Figure 8.** Concentration and Production of Lipid peroxidation and Hydrogen peroxide

Concentration and Production of Lipid peroxidation (MDA) and Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in common bean plants under maximum stress and rehydration condition, and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5\pm se$ ). T1-WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

Source: the author

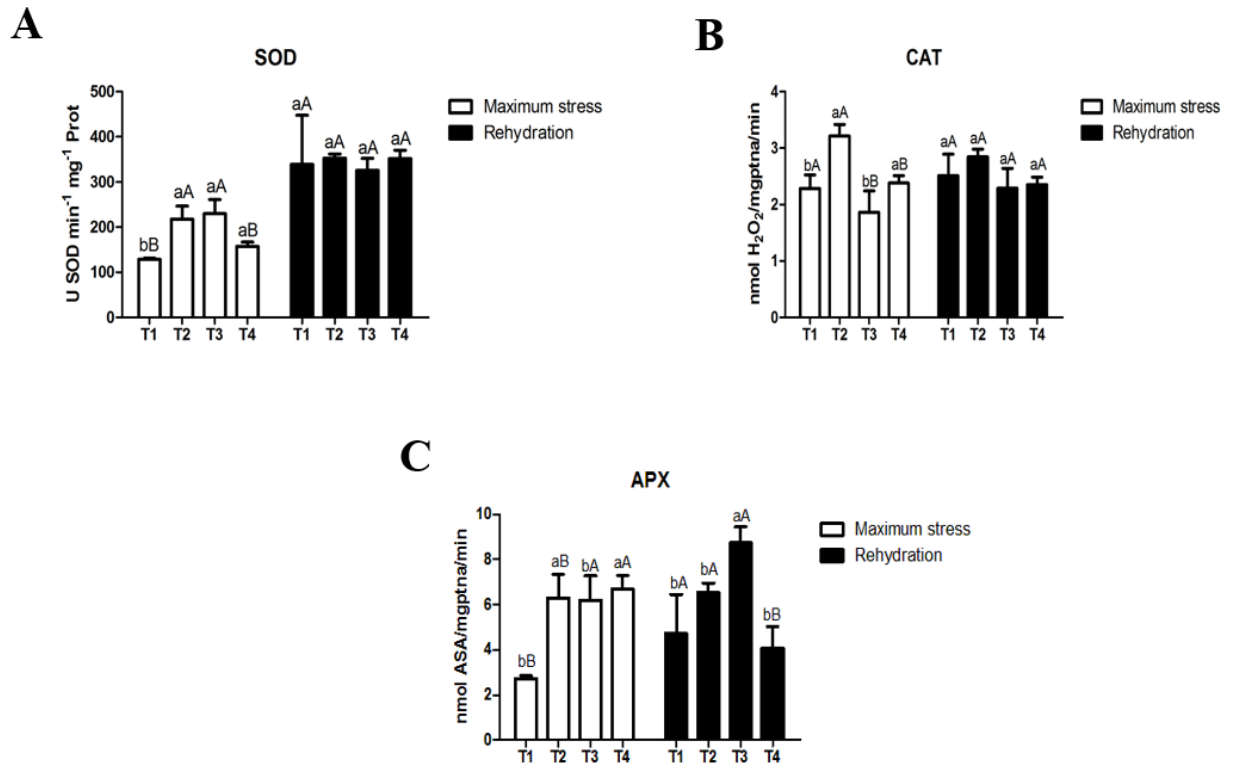
#### 5.4 Activities of Enzymes of the Antioxidant System under Maximum Stress and Rehydration Conditions

Among the specific activities of enzymes of the antioxidant system, it was observed that during maximum stress superoxide dismutase (SOD) had higher activity in T2 when compared with T4, and also T3 had higher activity compared with T1. However, all treatments were statistically similar during the rehydration period (Fig.8A).

The catalase (CAT) activity under maximum stress illustrated that T2 had higher activity compared to all the treatments. While in the return of irrigation, no statistical difference was observed in all the treatments (Fig.8B).

On the enzymatic activity of ascorbate peroxidase (APX), higher activity was observed in T4 when compared with T2 during the period of water stress. Under the rehydration condition, higher activity of APX was observed in T3 only (Fig.8C).

**Figure 9.** Enzymes activity of the antioxidant system



Enzymes activity of the antioxidant system of common bean plant under maximum stress and rehydration condition, and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5 \pm se$ ). T1-WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

Source: the author

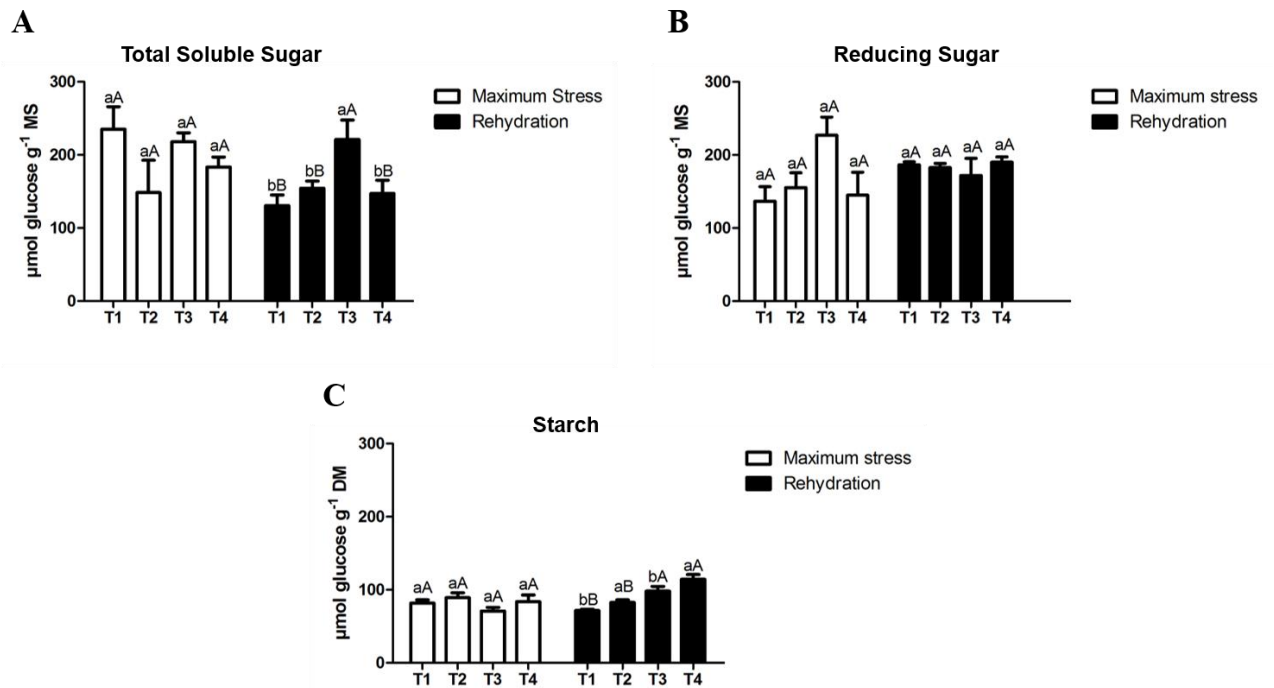
### 5.5 Effects of Vislumbre® on Total Soluble Sugars, Reducing Sugars and Starch on Common Bean Plants under Maximum Stress and Rehydration Conditions

During the period of water stress, total soluble sugars (TSS) results showed no statistical difference among all the treatments. However, a higher concentration of total soluble sugars (TSS) was observed in T3 compared with other treatments under rehydration conditions (Fig.9A).

Starch showed no significant difference between all the treatments during the period of maximum stress, while under rehydration conditions increased concentration of starch was observed in T4 as well the interaction between the factors showed a significant difference. (Fig.9B).

Reducing sugars (RS) results showed no statistical difference among all the treatments in both maximum stress and rehydration conditions (Fig.9C).

**Figure 10.** Concentration of total soluble sugars, Reducing sugars, and Starch



Concentration of total soluble sugars, Reducing sugars, and Starch in common bean plants common under maximum stress and rehydration condition, and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5 \pm se$ ). T1- WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

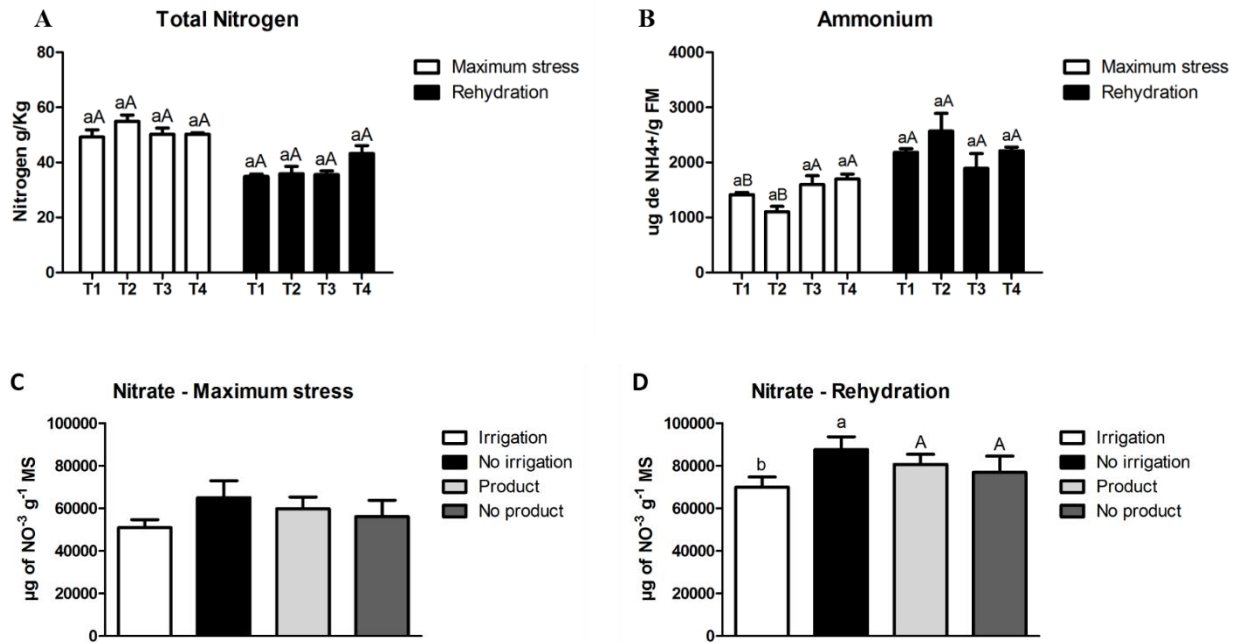
Source: the author

## 5.6 Effects of Vislumbre® on the Concentration of Total Nitrogen, Ammonium, and Nitrate in Common Bean Plants under Maximum Stress and Rehydration Conditions

During the period of both water stress and rehydration conditions, the total nitrogen result illustrated that all the treatments were statistically similar (Fig.10A). A higher concentration of ammonium was observed in T3 compared with T1. A similar concentration was also observed in T4 compared with T2 under maximum stress conditions. In rehydration

conditions, all treatments were statistically the same (Fig.10B). However, all the treatments means were similar in nitrate concentration during maximum stress (Fig.10C). Higher accumulation of nitrate was observed in treated plants during rehydration condition (Fig.10D).

**Figure 11.** Concentration of total nitrogen, ammonium, and nitrate



Concentration of total nitrogen, ammonium, and nitrate in common bean plants under maximum stress and rehydration conditions, and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5 \pm se$ ). T1-WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

Source: the author

### 5.7 Effects of Fertilizer on Gas exchange parameters on Common Bean Plants under Maximum Stress and Rehydration conditions.

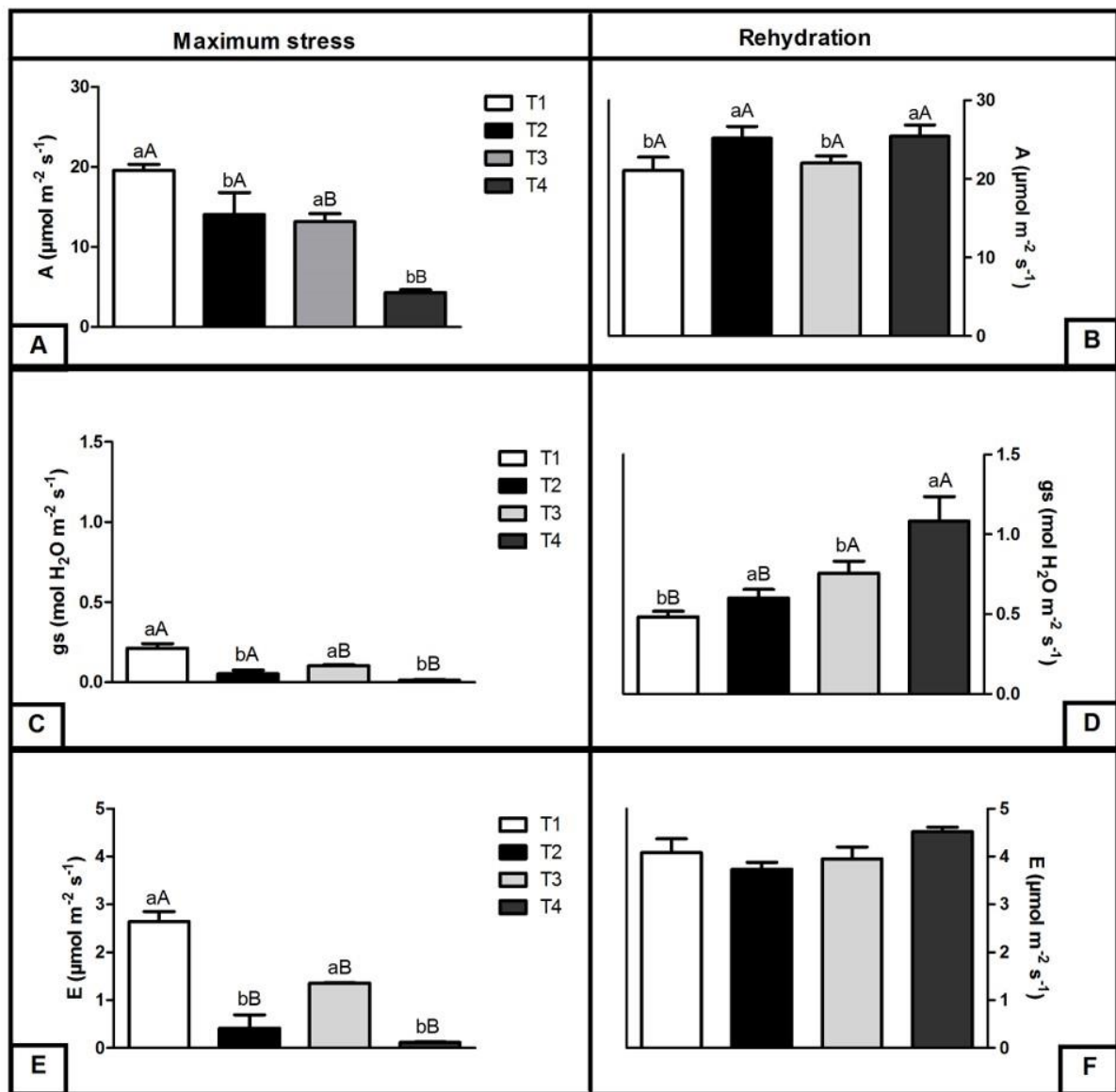
Gas exchange was significantly affected almost throughout the period of maximum stress in treatments with fertilizer application, irrespective of the condition. T1 showed significant increase in *A* when compared to T3, similarly, T2 was higher in *A* compared to T4 (Fig.11A). However, after resuming irrigation all the treatments recovered the gas exchange values without significant difference between T1 and T3, so also between T2 and T4. But T4 presented higher value when compared to T1 (Fig.11B).



Stomatal conductance ( $g_s$ ) increased substantially when compared with T3, while T4 showed a significant decline compared to T2 under the maximum stress condition (Fig.11C). In rehydration condition  $g_s$  values were higher in T3 compared to T1, and also T4 when compared to T2 (Fig.11D).

Additionally, T1 showed higher increased in  $E$  compared to T3, while T2 and T4 were statistically the same, although T4 presented lower value (Fig.11E). After resuming irrigation, all the treatments recovered the  $E$  value without significance difference between them (Fig.11F).

**Figure 12.** Net photosynthesis, stomatal conductance and transpiration



Net photosynthesis ( $A$ ), stomatal conductance ( $g_s$ ) and transpiration ( $E$ ) in common bean plants under maximum stress and rehydration conditions and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5\pm se$ ). T1-WW--

MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

Source: the author

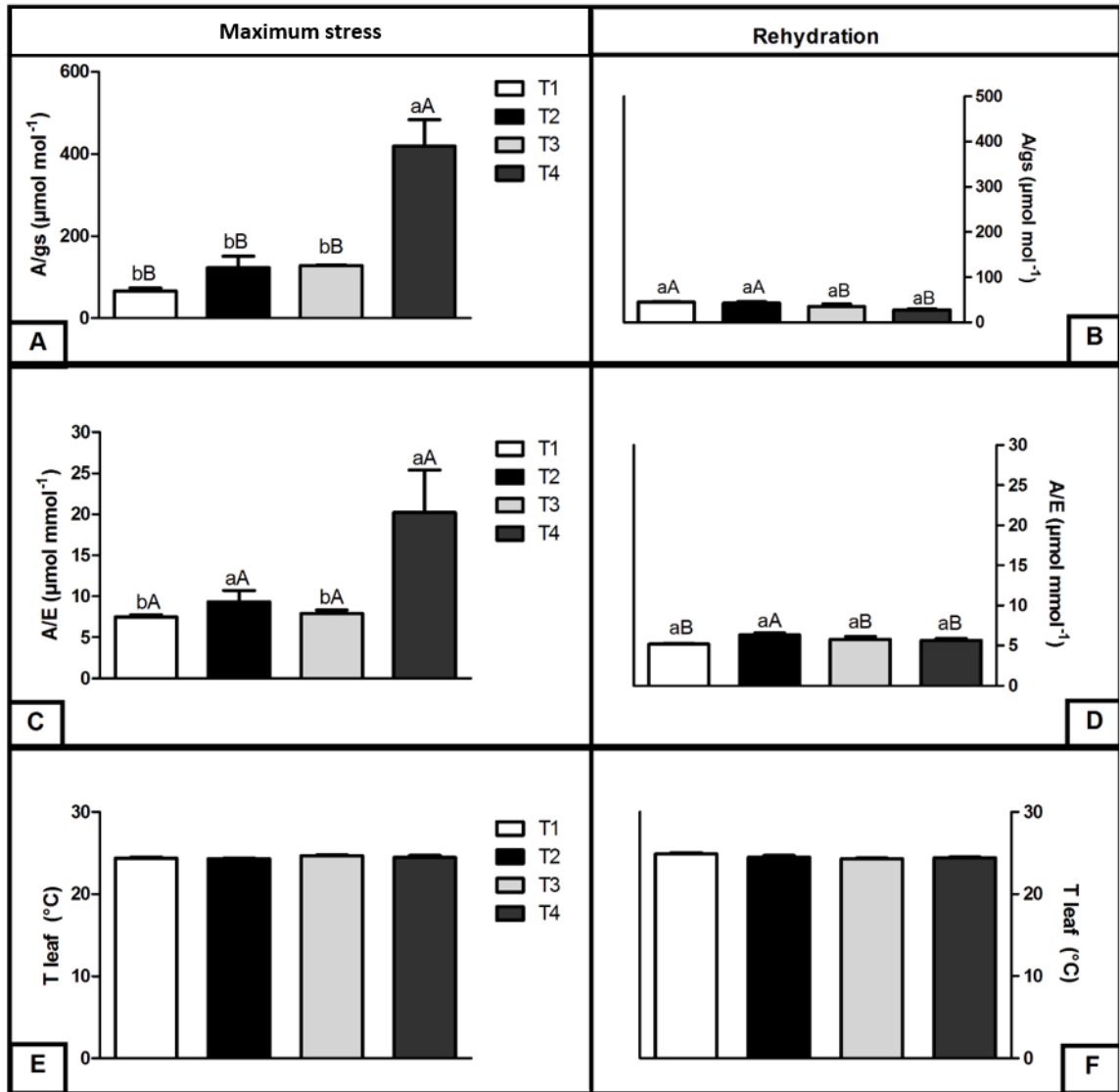
### **5.8 Effects of Fertilizer on Gas exchange parameters on Common Bean Plants under Maximum Stress and Rehydration conditions**

During the period of maximum stress, T4 showed a significant increase in (A/g<sub>s</sub>) value when compared to T2, while T1 and T3 shows no statistical difference though T3 present higher value than T1 (Fig.12A). Contrarily, T4 present lower value in (A/g<sub>s</sub>) with the return of irrigation compared to T2. Similarly, T1 presents was higher when compared with T3 under the same condition (Fig.12B).

Water use efficiency significantly increased in T4 compared to T3 and T1, but no statistical difference was observe compared to T2, although T4 presented higher water use efficiency under maximum stress condition. Additionally, no statistical differences were observed between T1 and T3 under the same condition (Fig.12C). More so, T2 present significant water use efficiency compared to all other treatments with the return of irrigation (Fig.12D).

Finally, all the treatments were statistically the similar regarding leaf temperature under both maximum stress and rehydration conditions (Fig.12E and F).

Figure 13. Water use efficiency , leaf temperature



Water use efficiency ( $A/E$ ), leaf temperature ( $T_{leaf}$ ) in common bean plants under maximum stress and rehydration conditions, and Vislumbre® application. Using the Scott-Knott test, different letters indicate statistical difference at a significance level of 5% ( $\leq 0.05$ ;  $n=5\pm se$ ). T1-WW--MOCK, T2- WD-MOCK, T3- WW-0.4 L ha<sup>-1</sup>, T4- WD-0.4 L ha<sup>-1</sup>. Capital letters evaluate the efficiency of the Vislumbre® and lowercase letters evaluate the influence of irrigation.

Source: the author

### **5.9 Morphological Measurements of Number of Mature Leaves, Number of non- mature Leaves, Number of Mature Flowers, Number of non- mature Flowers, Number of nodes, Leaf area of Mature and non- mature Leaves, Plant height and Diameter**

Regarding the number of mature leaves, only T4 and T3 displayed higher average values during rehydration conditions (Table 4). Similarly, T4 presented higher values compared to T2, with T3 showed lower values compared to T1 under same condition. However, the average values were similar in number of mature leaves under maximum stress condition (Table 4).

Under maximum stress conditions, T1 exhibited higher average values for non-mature leaves when compared with T3, similarly, T2 displayed higher average value compared to T4. During rehydration, T1 also presented higher average values compared to T3. Contrarily, T4 showed higher average values compared to T2 (Table 4).

It can be observed that the irrigation treatment T3 resulted in a higher average number of mature flowers compared to the non-irrigated treatments T1, T2, and T4 during maximum stress condition. During rehydration condition, T1 had shown the highest average number of mature flowers, while T3 had the lowest average. However, this indicates a potential recovery of T1 in flower production after stress (Table 4). With regards to the number of non-mature flowers, all treatments T1, T2, T3 have the same average, with exception of T4 that had slightly lower average (1.6) compared to the other treatments under maximum stress conditions. During rehydration period T1 presented the highest average compared to T3, while T4 had higher average when compared to T2 (Table 4).

For the number of nodes, T4 showed the highest average value compared to T2, similarly T3 presented higher values compared to T1 under maximum stress conditions. With resumption of irrigation, all the treatments displayed increased average value compared to maximum stress conditions. However, T4 showed higher average values compared to T2 and also T3 indicate higher values compared to T1 (Table 4).

With regards to leaf area for mature leaves, the results indicated that T3 and T4 had higher average values compared to T1 and T2 respectively under maximum stress conditions. However, all the treatments showed increased in average values during rehydration period, T1 displayed the highest average values of leaf area when compared to T3. Also, T2 presented higher average values compare to T4, indicating recovery potential (Table 4).

for leaf area of immature leaves T3 exhibited higher average values compared to T1, similarly T4 presented significant average values compared to T2 under stress condition. Furthermore, all the treatments demonstrated increased in average values with return of irrigation. Contrarily, T1 presented higher average value compared to T3, and also T2 had higher average value than T4 (Table 4).

Under maximum stress condition, T3 presented higher plant height average values compare to T1, similarly, T4 had little higher average values compared to T2. On the contrary during rehydration period, T1 exhibited higher average values when compared to T3. However, T4 displayed higher average values compared to T2 (Table 4).

Plant diameter result indicated higher average values in T1 compared to T3, also T4 exhibited higher average values compared to T2 respectively under maximum stress condition. Similarly, T1 present higher average value compared to T3 during rehydration period. Despite T2 increased average values during rehydration conditions, T4 showed higher values compared to it (Table 4).

Table 4- Morphological measurements of common bean plants under maximum stress and rehydration conditions

|                           | Treatments | Number of<br>mature leaves | Number of non-<br>mature leaves | Number of<br>mature flowers<br>(un.) | Number of<br>non-mature<br>flowers | Number<br>of nodes | Leaf area<br>(mature<br>leaves)<br>(cm <sup>2</sup> ) | Leaf area<br>(immature<br>leaves)<br>(cm <sup>2</sup> ) | Plant height<br>(cm) | Diameter<br>(mm) |
|---------------------------|------------|----------------------------|---------------------------------|--------------------------------------|------------------------------------|--------------------|---|---|----------------------|------------------|
| <i>Maximum<br/>stress</i> | T1         | 5.4 ±1.5                   | 2.8 ±1.3                        | 2.4 ±0.5                             | 2.2 ±0.4                           | 4.0 ±0.7           | 37.7 ±7.3   | 17.7 ±5.7   | 27.8 ±3.1            | 5.2 ±0.2         |
|                           | T2         | 5.2 ±0.8                   | 3.0 ±1.0                        | 2.2 ±1.5                             | 2.2 ±1.3                           | 4.6 ±0.5           | 32.3 ±12.8  | 15.1 ±5.2   | 27.6 ±1.5            | 3.8 ±0.1         |
|                           | T3         | 5.6 ±0.9                   | 2.4 ±1.5                        | 3.4 ±0.9                             | 2.2 ±0.8                           | 4.6 ±0.5           | 40.0 ±8.4   | 19.0 ±2.7   | 30.6 ±0.9            | 4.2 ±0.4         |
|                           | T4         | 4.4 ±1.1                   | 2.4 ±0.9                        | 2.8 ±0.4                             | 1.6 ±0.9                           | 4.8 ±0.4           | 40.1 ±3.9   | 20.5 ±3.1   | 28.3 ±3.2            | 4.6 ±0.4         |
| <i>Rehydration</i>        | T1         | 6.8 ±0.8                   | 3.6 ±0.9                        | 5.2 ±1.5                             | 2.4 ±1.3                           | 6.2 ±0.4           | 73.5 ±9.7   | 37.1 ±16.4  | 30.4 ±2.6            | 5.3 ±0.2         |
|                           | T2         | 6.0 ±1.0                   | 2.2 ±1.1                        | 4.8 ±2.2                             | 0.8 ±0.4                           | 6.8 ±1.3           | 63.6 ±15.8  | 34.3 ±10.9  | 29.2 ±3.7            | 4.4 ±0.3         |
|                           | T3         | 8.0 ±2.2                   | 1.8 ±1.7                        | 3.1 ±1.5                             | 1.0 ±0.0                           | 7.0 ±1.2           | 59.5 ±17.5  | 36.3 ±19.4  | 29.9 ±2.2            | 4.5 ±0.4         |
|                           | T4         | 8.4 ±0.5                   | 3.6 ±1.8                        | 4.6 ±1.5                             | 1.8 ±0.8                           | 7.0 ±1.4           | 62.7 ±16.3  | 29.8 ±12.6  | 30.8 ±0.6            | 4.8 ±0.3         |

The data represent the averages of five (5) replication. Values show mean ± sd (n = 5)

### **5.10 Effects of Fertilizer on Chlorophylls *a* and *b* Relative Water Contents, and Leaf Water Potential under Maximum Stress and Rehydration Conditions.**

During the maximum stress period, there were no much differences observed for chlorophyll *a* among all the treatments, but, T4 showed little higher value compared to T2. More so, recovery condition led to increase in chlorophyll *a* content in all the treatments with similar average values shown among the treatments. However, T1 present little higher average values compared to T3 and also T2 displayed little higher average values compared to T4 (Table 5).

Regarding Chlorophyll *b*, there were no much differences observed among the treatments under maximum stress condition. But, T1 showed little higher average values compare to T3, similarly, T4 exhibited higher little mean values compared to T2 respectively. During rehydration condition, T1 had shown higher mean value compared to T3. On the contrary, T4 presented lower average values compared to T2 (Table 5).

With the exception of T4, which drastically reduced relative water content values under maximum stress, the leaf relative water content (RWC) increased in all of the treatments. When irrigation resumed, T4's RWC values were restored as opposed to T2, which noticeably had decreased RWC values. Additionally, RWC values for T3 were higher than T1, which dramatically decreased (Table 5).

Under conditions of maximum stress, T4 showed higher values for the pre-dawn leaf water potential ( $\Psi_{pd}$ ) than T2, while T3 also shown higher values than T1. However, in the rehydration condition, all of the treatments showed greater leaf water potential values ( $\Psi_{pd}$ ), with T3 showing significantly higher values than T1 and T4 showing significantly higher values than T2 (Table 5).

Table 5- Photosynthetic pigments, Relative water content and leaf water potential measurements

|                       | Treatment | Chlorophyll <i>a</i><br>(dimensionless) | Chlorophyll <i>b</i><br>(dimensionless) | Relative water<br>content (%) | Leaf water<br>potential<br>(KPa) |
|-----------------------|-----------|---|---|-------------------------------|----------------------------------|
| <i>Maximum stress</i> | T1        | 35.0 ± 2.1                              | 11.0 ± 1.5                              | 72 ± 9                        | -0.26 ± 0.10                     |
|                       | T2        | 34.5 ± 0.5                              | 10.6 ± 0.7                              | 71 ± 12                       | -0.35 ± 0.10                     |
|                       | T3        | 35.2 ± 1.5                              | 10.7 ± 0.9                              | 67 ± 9                        | -0.29 ± 0.10                     |
|                       | T4        | 35.1 ± 1.5                              | 11.3 ± 1.6                              | 58 ± 8                        | -0.44 ± 0.10                     |
| <i>Rehydration</i>    | T1        | 37.2 ± 1.5                              | 12.4 ± 1.2                              | 61 ± 3                        | -0.46 ± 0.10                     |
|                       | T2        | 37.0 ± 1.3                              | 12.1 ± 1.3                              | 56 ± 3                        | -0.33 ± 0.10                     |
|                       | T3        | 36.3 ± 1.2                              | 11.1 ± 1.3                              | 68 ± 6                        | -0.73 ± 0.40                     |
|                       | T4        | 36.7 ± 0.1                              | 11.8 ± 0.7                              | 66 ± 6                        | -0.61 ± 0.20                     |

The data represent the averages of five (5) repetitions. Values show mean ± sd (n = 5)

### 5.11 Effects of Leaf -to-air Vapor pressure deficit (VPD<sub>L</sub>) and Temperature in Common bean during Maximum and Rehydration condition.

All the treatments showed a greater increment in leaf-to- air vapor pressure deficit (VPD<sub>L</sub>) under maximum stress conditions. However, although no statistical difference was observed between the treatments, T4 had higher value than T2. So also increased values were shown in T3 than T1 while air temperature remained constant.

A significant similar decrease in leaf -to -air vapor pressure deposit (VPD<sub>L</sub>) was observed in all the treatments while air temperature remained constant during rehydration condition (Table 6).



Table 6- Leaf -to-air Vapor pressure deficit (VPD<sub>L</sub>) and Temperature in Common bean during Maximum and Rehydration condition

| PARAMETERS     | MAXIMUM STRESS |            |            |            | REHYDRATION |            |            |            |
|----------------|----------------|------------|------------|------------|-------------|------------|------------|------------|
|                | T1             | T2         | T3         | T4         | T1          | T2         | T3         | T4         |
| VpdL (kPa) out | 1.1 ± 0.1      | 1.2 ± 0.1  | 1.3 ± 0.9  | 1.3 ± 0.0  | 0.9 ± 0.1   | 0.7 ± 0.0  | 0.7 ± 0.1  | 0.7 ± 0.1  |
| Tair (°C) in   | 24.9 ± 0.1     | 24.9 ± 0.0 | 25.3 ± 0.1 | 24.9 ± 0.1 | 25.1 ± 0.1  | 25.2 ± 0.2 | 25.1 ± 0.1 | 25.2 ± 0.1 |

The data represent the averages of five (5) repetitions. Values show mean ± SE (n = 5)

## 6. DISCUSSION

Drought-induced water stress is one of the most dangerous environmental stressors for plant growth because it significantly decreases the growth, development, and productivity of agricultural crops (SALEM et al., 2022) EL–METWALLY; GERIES; SAUDY, 2022). In this study, we observed mass reduction in grains number, seed weight and number of pods in common bean plants subjected to water deficit condition when compared with irrigated plant (FIG..1A, B and C). However, when compared to treated plants, we established that the foliar application of Vislumbre® increased common bean plant tolerance under water deficit conditions. This occurred as a result of Vislumbre®'s improved stomatal conductance, transpiration, and water use efficiency, which caused more pods and grains to be produced at the end of the experiments when compared T4 to T2. While T3 and T1 were statistically similar. This indicated that the fertilizer had no effect under irrigated condition. With regards to number of pods, T1 presented significant increased compared to T3, this also showed non – effect of the fertilizer under irrigated condition. Contrarily, T4 showed significant increased in number of pods compared to T2. This increased in pods number was attributed to the influence of the fertilizer.

These results showed fertilizer effectiveness at alleviating the negative effects of water stress condition as reported by (MA; FREITAS; DIAS, 2022), that amino acid enriched fertilizer can improve plant growth and yield performance unde drought stress condition. Similarly, (FERREIRA et al., 2022), reported that fertilizer application played important role in the bean plant development, by making it more resistant to environmental stresses. Our findings also correlate with that of (ABRANTES et al., 2011), who observed that the application of growth

stimulant increased the number of grains per plant and grain yield in common bean under cerrado conditions. Similarly, (MAMBRIN et al., 2020), discovered that the supplementation in the bean crop promotes positive responses of dry mass of pods, number of pods, number of grains and grain yield. Moreover, the increased grains yield observed in this study for plants treated with fertilizers is associated with improved plants tolerance to abiotic stress condition, which according to (BATTACHARYYA et al., 2015) could be attributed to various protective strategies such as the regulation of related genes, accumulation of osmolytes, improvement in water use efficiency and other effects on the plant rhizosphere.

The seed mass (FIG.1C) on the contrary, no statistical difference was observed among the treatments. However, T3 presented higher value compared to T1, so also T2 showed increased seed mass compared to T2. This indicated that the fertilizer had increased seed mass under irrigated condition. The lower value presented by T4 compared to T2, could be due to the application of fertilizer at non appropriate growing stage of the plant. Our result correlated with the findings of (CARVALHO et al., 2022) who discovered that fertilizers applications at R5 stage promoted the best results for number of non-commercial grains, and thousands of grains mass of common beans cultivars evaluated.

Regarding the seed weight (FIG.1D). All treatments were statistically the same. This means that the fertilizer do not increased seed weight in all the conditions. This could also be attributed to plant growing stage application of the fertilizers. The result correlate with the findings of (LANA et al., 2009) found increased in the production of bean grains with fertilizer but the same did not influence the weight of a thousand grains.

The total amino acids (AA), and proline (Pro), concentration was higher in T4 than T2 as well as T3 compared to T1 in common bean leaves under drought stress condition (FIG.3A and B). That is, fertilizer favored greater synthesis of amino acid and consequently proline in T4 under drought stress. The amino acids forms under T4 can be used as a source of nitrogen for protein synthesis, as well as they can be used in the energy supply for plants to recover more quickly from the water stress condition. The amino acid increase in response to water deficits may be due to a decrease in protein synthesis, or a greater protein degradation. However, the free amino acid increase, mainly proline, functions as a molecular chaperone by stabilizing proteins and preventing their misfolding and aggregation in addition to contributing to osmotic adjustments in tissue by functioning as an inert, compatible osmotic agent (GHOSH et al., 2022). Proline has diverse roles in stress conditions, such as maintaining cell turgor, as a source of carbon and nitrogen, chelation of metals, and as a signaling molecule (DE FREITAS et al., 2018; AGHDAM et al., 2019). Because they are significant byproduct of the metabolism of

nitrogen, Proline and amino acid can be crucial organic solutes in protecting cells from harm brought on by drought stress (ANDRADE et al., 2016; RAO; CHAITANYA, 2016).

Regarding the accumulation of amino acids and proline in non-stress plant T3 (FIG.3A and B). This could be due to present of substantial amount of nitrogen in the fertilizer formulation, since nitrogen (N) is a component of amino acids, the building blocks of proteins and enzymes. (MATTIOLI; FRANCIOSO; TROVATO, 2022), reported that proline is also synthesized in unstressed environments, where it modulates the floral transition. The decrease in proline and amino acid during rehydration may possibly due to restoration of osmotic adjustment and helps prevents the build-up of ROS and reduces oxidative stress (SHARMA et al., 2019a).

The high accumulation of protein in T1 under maximum stress (FIG.3C) than all other treatments was probably due to RUBISCO activity as a result of increased photosynthetic activity in leaves as indicated in the gas exchange data in which T1 had higher net photosynthesis accumulation, possibly because the plant was not under stress as previous findings attributed decreased RUBISCO activity under drought stress in plants. This correlated with findings of HAWORTH et al., (2019) who stated that because CO<sub>2</sub> is the substrate for plant photosynthesis, drought frequently lowers net photosynthetic rate which is accompanied by increased CO<sub>2</sub> diffusion resistance and decreased activity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in leaves .

The capacity of the plants to accumulate total nitrogen in all the treatments irrespective of water or fertilizer application during and after the stress (FIG.8A) could probably due to the leguminous nature of the common bean plant having high nitrogen content and ability to interact with symbiosis bacteria for biological nitrogen fixation. As reported by BESHIR et al., (2015), common bean can interact with Rhizobium bacteria to fix more than 160 kg of atmospheric nitrogen per hectare into the soil. Similarly, PACHECO et al., (2020) reported that due to common bean high fixation capability when compared to other leguminous species, this element (N) can be provided to the soil by symbiotic nitrogen fixation. The increased accumulation of ammonium uptake observed in T4 compared to T2, and T3 compared to T1(FIG.8B) was possibly as a result of overexpression of nitrate reductase (NR) or nitrite reductase (NiR) as they actively involved in the nitrogen assimilation pathways, specifically in the conversion of NO<sub>2</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup>. The accumulation of NO<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the nitrogen metabolism process is essential for maintaining the activities of key enzymes, such as GS and GOGAT for plant growth and development (NGUYEN et al., 2005). NH<sub>4</sub><sup>+</sup> is converted to glutamate and glutamine through the glutamine synthetase (GS), glutamate synthetase

(GOGAT) (XU; ZHOU, 2006). Important metabolic indicators of drought tolerance are thought to be the activity of GS and GOGAT (NAGY et al., 2013; SINGH; GHOSH, 2013). We can say that the high uptake of ammonium in T4 was to improve drought tolerance while that of T3 has to do with nitrogen content in the fertilizer. However, no statistical difference was observed during rehydration signified plant's ability to take up and distribute nutrients including ammonium.

Furthermore, the observed similar concentration of nitrate observed in all the treatments under maximum stress condition might possibly due to nitrate metabolism regulation. Plants have regulatory mechanisms to maintain nitrate homeostasis, which involves balancing nitrate uptake, assimilation, and storage (CHAMIZO-AMPUDIA et al., 2017). These regulatory mechanisms might function similarly in both treated and untreated plants under drought conditions, resulting in comparable nitrate concentrations. Additionally, the observed accumulation of nitrate in fertilizer treated plants compared to non-treated ones could due to improved nitrogen assimilation. The availability of nitrogen from the fertilizer could have promoted the synthesis of nitrate-producing enzymes and proteins involved in nitrate assimilation, resulting to more efficient conversion of nitrate into amino acids and other nitrogen-containing compounds (LIU; HU; CHU, 2022). Numerous enzymes, including glutamine synthetase (GS), nitrate reductase (NR), nitrite reductase (NiR), and glutamine-2-oxoglutarate aminotransferase (GOGAT, also known as glutamate synthase), are involved in the process of nitrate assimilation (LIU; HU; CHU, 2022).

The negative effect of ROS on membranes is manifested as an increased MDA concentration in T4 compared to T2, and T3 when compared to T1 resulting in lowering membrane stability as MDA could react with free amino groups of proteins and phospholipids components (KOCHEVA et al., 2014). The higher concentration of MDA in T3 and T4 may possibly due the nitrogen metabolism and assimilation as increased nitrogen availability from amino acid fertilizer can enhance nitrogen metabolism and assimilation in plants. However, nitrogen metabolism can produce byproducts such as nitric oxide (NO) and ammonium ions (NH<sub>4</sub><sup>+</sup>), which can contribute to oxidative stress and the accumulation of ROS. Excess ROS can promote lipid peroxidation and the subsequent formation of MDA (MORALES; MUNNÉ-BOSCH, 2019). (Figure 4 and 5A). However, upon irrigation resumption, the plants showed a strategy of repair as a result of the synthesis of compounds such as amino acids and proline. Plants accumulates numerous metabolites such as sugars, organic acids and amino acids in response to stress (RAZA et al., 2023).

ROS are frequently formed as metabolic byproducts when stressed plants grow (MEDINA et al., 2021). Superoxide, hydrogen peroxide, hydroxyl, and singlet oxygen are examples of ROS that are produced when electrons from the electron transport chain in mitochondria and chloroplasts leak and react with the oxygen molecule without the assistance of any other electron acceptors (PHUA et al., 2021). The accumulation of reactive oxygen species (ROS) results in oxidative damage to plants' nucleic acids, proteins, and lipids as well as the breakdown of chlorophyll pigments (EL-BELTAGI et al., 2020). The increase in the steady-state level of hydrogen peroxide found in all treatments, regardless of the application of a fertilizer or water, was a sign that the balance of the cells had shifted in favor of oxidative stress (MILLER et al., 2010). The observed rise in hydrogen peroxide levels in T1 under maximum stress condition, could be due to endogenous factors, because disruption or imbalance in the antioxidant system, such as decrease in the enzyme activity or a deficiency in non-enzymatic antioxidants can lead to elevated ROS levels (AGUILAR; NAVARRO; PÉREZ, 2016). While that of T3, could probably be attributed to nitrogen metabolism. Increased nitrogen availability from the amino acid fertilizer applied to T3 may have stimulated nitrogen metabolism, leading to the generation of ROS. Nitrogen metabolism can produce byproducts such as nitric oxide (NO) and ammonium ions (NH<sub>4</sub><sup>+</sup>), which can contribute to oxidative stress and the accumulation of ROS (MORALES; MUNNÉ-BOSCH, 2019). However, upon rehydration, the hydrogen peroxide levels returned to normal in T1 compared to T3, and similarly in T2 compared to T4. The high levels of hydrogen peroxide observed in plants treated with fertilizers during rehydration period, could be due to activation of stress response pathways because drought stress followed by rehydration can activate stress response pathways in plants (DONG et al., 2019). These pathways can involve the production of ROS as signaling molecules to activate defense mechanisms (SHARMA et al., 2019b). On top of that, ROS play an important role for plants to adapt to a variety of abiotic stressors (SHARMA et al., 2019b).

Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) are antioxidant enzymes that assist plants tolerate drought stress by scavenging ROS and shielding the plant cells from oxidative damage (MOURSI et al., 2021).

The lower activity of superoxide dismutase (SOD) observed in T4 under maximum stress could be due to fertilizer effects in improving plant tolerance to drought stress. By boosting antioxidant defense and expanding nitrogen uptake, as nitrogen supplies could lessen the negative impacts of drought stress on plants while decreasing the activity of SOD (LI et al., 2020a). With regards to increased SOD activity in T3 with is non stress plant could be attributed to enhanced nitrogen metabolism as amino acid fertilizer can provide additional source of

nitrogen for the plants which can lead to increase metabolic activity. This increased metabolic activity can stimulate the production of superoxide radicals as byproducts which triggers a compensatory response in form of SOD activity. Our finding correlate with that of (HAYYAN; HASHIM; ALNASHEF, 2016), who proposed that superoxide radicals might be produced as a consequence of elevated metabolic activity, and that this elevated metabolic activity could be encouraged by an additional source of nitrogen for plants. Also, high activity of SOD witnessed in T2 probably demonstrated the plants' tolerance to the increased ROS brought on by drought stress. SOD is widely distributed in aerobic organisms and serves as a catalyst in the conversion of superoxide into oxygen and molecular hydrogen peroxide ( $H_2O_2$ ) (CHEN et al., 2022). ABID et al., (2018) reported increased activity of SOD in wheat leaves under drought stress. The high activity of SOD observed in all the treatments during rehydration period could be attributed to water that help to improve tolerance to the plants. This correlated with findings of DONG et al., (2019) who reported increased activity of SOD in soy bean plant during rehydration period after stress condition.

The high activity of catalase (CAT) witnessed in T2 indicates plant high tolerance level of drought stress by reducing the formation ROS and lowering the oxidative damage through continuously converting hydrogen peroxide ( $H_2O_2$ ) in to water (WANG et al., 2018). Furthermore, the low activity of CAT observed in T4 against T2, it is possible that the amino acid fertilizer have caused increase in nitrogen metabolism which could have led to a decrease in catalase (CAT) activity. By increasing nitrogen metabolism and antioxidant defense, amino acid fertilizers with nitrogen can help plants cope with drought stress (AHANGER et al., 2021). The high activity of CAT witnessed in all the treatments during rehydration could be attributed to balance between ROS production and antioxidant production (WANG et al., 2018).

Ascorbate peroxidase (APX) high activity observed in T4 under drought stress indicate the amino acid fertilizer increased the activity of APX there by increasing the antioxidant capacity of the plant, highlighting the role of nitrogen metabolism in reducing drought stress. This correlate with findings of SUN et al., (2015), who stated that application of amino acid fertilizer containing nitrogen resulted in increased APX activity. The high activity of APX observed in T2 could be due to plant ability in tolerance to drought stress as APX involved in detoxification of hydrogen peroxide by converting it in to water (WANG et al., 2018). The low activity of APX witnessed in T4 during rehydration could be due recovery of the plants from the stress.

The high increased in leaf-to-air vapor pressure deposit witnessed during maximum stress condition in all the treatments could be attributed to high environmental temperature

couple with low humidity in the plant growing environment on the data collection day. creating a stressful environment for the plants. Our findings correlate with that of (GROSSIORD et al., 2020) who reported increased  $VPD_L$  due to increased temperature.

A decrease in stomatal conductance for all the treatments under maximum stress may possibly due to increase  $VPD_L$ . As reported by (GROSSIORD et al., 2020) Stomatal conductance,  $g_s$ , and steady-state stomatal aperture often decrease when  $VPD_L$  increases quickly.

Additionally, the decreased stomatal conductance and transpiration level and also increased level of intrinsic water use efficiency ( $A/g_s$ ) and water use efficiency (WUE) observed in T4 under maximum stress could be attributed to effect of amino acid fertilizer by influencing hormonal balance. By promoting the production of stress-related hormones like abscisic acid (ABA), which is essential for controlling stomatal closure and minimizing water loss, amino acid fertilizers can affect the hormonal balance in plants. Amino acid fertilizers can raise WUE and promote plant responses to drought stress by altering hormone levels and osmotic potential (DOS SANTOS et al., 2022).

However, the observed increased transpiration level during rehydration for all the treatments, may likely linked to decreased  $VPD_L$  during rehydration condition as a function of stomatal regulation. When  $VPD_L$  is low and stomata are fully open, leaf level transpiration (T) increases linearly with  $VPD_L$ . This is because stomatal regulation directly controls T (GROSSIORD et al., 2020).

soluble sugars, In addition to serving as nutrients, have important osmoprotective properties that work to shield plant cells from the harm resulting from drought stress (LI et al., 2020b).

The starch result indicates that drought stress did not affects the treatments. Plants use starch to increase tolerance to stress by converting starch into glucose, known as starch degradation, for plants to get their energy. The generated glucose can be utilized to sustain and promote cellular development (HASAN et al., 2023). The accumulation of starch in both amino acid fertilizer treated treatments during rehydration period, showed ability of the fertilizer to produce Osmo protectants.

The accumulation of total soluble sugars under maximum stress condition, may possibly due to osmotic adjustment by the plants. (SADDHE; MANUKA; PENNA, 2021) reported Sugars play an important role in the perception of stress, in stress signaling, and as a regulatory hub for stress-mediated gene expression, ensuring responses such as osmotic adjustment, scavenging of reactive oxygen species, and maintaining the cellular energy status through

carbon partitioning. The accumulation of total soluble sugars in non-stress plants, could be due to storage of energy to be use in the future. This correlated with the findings of (AFZAL; CHAUDHARY; SINGH, 2021). The accumulation of total soluble sugars in non- stress plant treated with amino acid fertilizer could be attributed to nitrogen presence in the formulation of amino acid fertilizer which consequently leads to increased photosynthesis and carbon fixation. Under non-stress conditions, plants treated with amino acids enriched fertilizer accumulate total soluble sugars as a result of improved photosynthesis and carbon fixation. Some amino acids, like tryptophan and glycine, can improve plant photosynthesis and carbon fixation (MOSA; ALI; ABDELSALAM, 2021).

The accumulation of reducing sugars observed in stress under maximum stress plants could be due to osmotic adjustment, this is due to the fact that during drought conditions, reducing sugars, an osmotically active solute, can accumulate in plant cells. Increased osmotic potential and water absorption by plant cells can result from sugar accumulation (AHLUWALIA; SINGH; BHATIA, 2021). The accumulation of reducing sugars in non-stress plants, could be due to storage of energy to be use in the future. Regarding the accumulation of reducing sugars under rehydration condition, this could be attributed to the increased carbohydrate metabolism activity during drought stress, which includes noteworthy increases in the activities of sugar metabolism enzymes and expression levels of GmSPS, GmSuSy, GmC-INV, GmA-INV, GmAMY3, and GmBAM (DU et al., 2020).

Photosynthesis and plant growth depend on photosynthetic pigments for light harvesting (MA; DIAS; FREITAS, 2020). The little increased in chlorophyll *a* and *b* contents observed in T3 compared to T1 and T4 against T2 under maximum stress condition and decreased in the concentration of these pigments during rehydration condition could be as a result of shift in the investment of nitrogen for the synthesis of osmoprotectants such as proline and amino acids for ameliorating the effects of drought stress by the plants. Proline and amino acids, which are important byproducts of the nitrogen metabolism, can be essential organic solutes for defending cells against damage caused by drought stress (ANDRADE et al., 2016).

The relative water content (RWC) can be used to assess the degree to which a plant is managing its water balance. RWC is associated with water potential and can be employed to monitor the water status of plants (GONZÁLEZ; GONZÁLEZ-VILAR, 2001). The relative water content demonstrated an important connection to gas exchange as it exert its influence on plants under maximum stress conditions. Dynamic changes in photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*) and RWC have extensively studied under maximum stress conditions. The observed decreased in photosynthesis in T3 compared to T1, T4 compared to



T2, and lowered stomatal conductance in T3 compared to T1, T4 against T2 and also decreased transpiration rates in T3 compared to T1 and T4 compared to T2 was possibly due to stomatal closure as influenced by nitrogen metabolism sequel to application of amino acid fertilizer which led to stomatal closure. Stomatal closure during drought stress plays a major role in water conservation by lowering transpiration rates and maintaining the relative water content of the plant (AHMAD, 2016). Additionally, the higher increase in RWC T3 compared to T1 and T4 compared to T2 following rehydration could be linked to the effect of amino acid fertilizer through nitrogen metabolism. Under drought stress, nitrogen fertilizer application raised the water content of several plant parts (XU et al., 2022).

Water potential ( $\Psi$ ) as a measure for monitoring track of a plant's water condition, such as the relative water content (LIMA et al., 2002), indicated a strong connection to gas exchange as water potential serves as signals of soil moisture levels, and maintaining this potential is crucial for the health of the plant's vascular system (AHLUWALIA; SINGH; BHATIA, 2021). The lower water potential observed in T3 compared to T1, and T4 when compared with T2 under maximum stress conditions could be attributed to closing of stomata which was influenced by the amino acid enriched fertilizer due to presence of nitrogen. This happens when nitrate enters the cell, and high levels of potassium, chloride, sucrose, malate, and nitrate in the cytosol cause the osmosis of water into the guard cells. As a result of the increased turgor pressure, the guard cells stretch and bend, opening the stoma (MORROW MELISSA HA; ALGIERS, 2022). Nitrogen can influence stomatal behavior and regulate the opening and closing of stomata (ZHU et al., 2020). Low water potential ( $\Psi$ ) witnessed in T3 compared to T1 and T4 compared to T2 during rehydration condition could be due to physiological changes triggered by the fertilizer which may have altered the plant's ability to reestablish its water potential quickly after rehydration as nitrogen can influence specific responses in plants, such as influence of hormone levels and signaling pathways (VEGA; O'BRIEN; GUTIÉRREZ, 2019).

Morphological data regarding number of matured leaves under maximum stress condition showed lower number of matured leaves in T4 compared to T2, this could be attributed to amino acid enriched fertilizer which might have influenced the production and signaling of abscisic acid (ABA), leading to stress responses (MUHAMMAD ASLAM et al., 2022). While the increment observed in the number of matured leaves during rehydration period in T3 compared to T1, and T4 compared to T2 may possibly attributed to hormonal regulation as nitrogen present in the fertilizer might have modulated hormonal balance of auxins and

cytokinin there by promoting leaf growth leading to a higher number of mature leaves (VEGA; O'BRIEN; GUTIÉRREZ, 2019).

Result of non-mature leaves indicated lower number in T3 compared to T1, and T4 when compared to T2 under maximum stress condition, was likely due to the amino acid enriched fertilizer treatment which might have improved water uptake and utilization efficiency in the treated plants, allowing them to maintain better hydration and minimize water stress. Improved water use efficiency can contribute to reduced leaf loss and a lower number of non-mature leaves under drought conditions (FAROOQ et al., 2009). The increment of non-mature leaves observed in T4, compared to T2 during rehydration condition could be related to hormonal balance disruption. The application of amino acid enriched fertilizer might have disrupted the hormonal balance, leading to a delayed or altered maturation process in these plants. This disruption could have contributed to the increase in non-mature leaves. Hormone content variations that are significant can be detrimental to plant growth and development. (PELEG; BLUMWALD, 2011). Additionally, decreased in non-mature leaves witnessed in T3 compared to T1 could be due to allocation of more resources towards leaf development, resulting in a higher proportion of mature leaves and a reduced number of non-mature leaves, since the fertilizer contained nitrogen which can promote vegetative growth in plants. FATHI, (2022), Stated that sufficient nitrogen content can support plant growth through photosynthesis.

Number of mature flowers increased in T3 compared to T1 under maximum stress condition might be related to increase nutrient partitioning influenced by the fertilizer through provision of an additional nitrogen source, which promoted nutrient allocation towards flower buds leading to increased number of mature flowers. Nitrate ions are utilized to synthesize amino acids and other nitrogen-containing chemicals essential for plant growth and development, including flower formation (LIU; HU; CHU, 2022). The observed increased in T1 compared to T3 during rehydration could be attributed to carbon-nitrogen balance, thus, non-treated plants might have maintained a more optimal carbon-nitrogen balance, favoring flower production and resulting in higher number of flowers. An imbalance in the carbon-nitrogen ratio can lead to changes in carbohydrate metabolism, nutrient assimilation, and energy allocation within the plant (REYES et al., 2016), which might have potentially affected flower development in treated plants.

The observed decreased in number of non-mature flowers in T4 when compared to T2 under maximum stress condition could be attributed to stress response and reproductive allocation. Because under maximum stress condition plants prioritize resources towards

survival and stress tolerance rather than reproductive growth. The application of the amino acid enriched fertilizer might have improved the stress tolerance of the treated plants, leading to a higher allocation of resources towards stress defense mechanisms rather than flower production. As a result, the treated plants may have invested fewer resources into the formation of non-mature flowers compared to non-treated plants. Through osmotic adjustment and photosystem protection, nitrogen can increase plants' ability to withstand drought (DING et al., 2018). The increased in number of non-mature flowers witnessed in T4 compared to T2, during rehydration conditions could be attributed to enhanced recovery and metabolic processes. The fertilizer might have facilitated the recovery and metabolic processes in the treated plants. availability of nitrogen from the fertilizer could have provided the necessary resources for the plants to recover quickly from the previous stress period and resume normal physiological functions, including flower development. Physiological processes such as nitrate or ammonium transport via cell membranes in roots, nitrate reduction in roots and leaves, N<sub>2</sub> fixation within nodules for legumes, and ammonium assimilation regulate a plant's ability to use nitrogen (DUBEY; SRIVASTAVA; PESSARAKLI, 2021). In addition, the decreased in non-mature flowers observed in T3 compared to T1 during rehydration might be due to resource allocation. It is possible that the treated plants prioritized the utilization of nitrogen for other growth and recovery processes, resulting in a reduced allocation of resources to non-mature flower development (TANG et al., 2021).

Furthermore, the witnessed increment in number of nodes in T3 compared to T1 during rehydration period might be related to hormonal effects as nitrogen availability in the fertilizer can influence hormonal regulation within the plants which promoted increased in cell division and elongation, leading to the development of more nodes. Plant hormones including auxins, cytokinin, and gibberellins drive plant cell division, elongation, and differentiation, which results in an increase in nodes (MORROW MELISSA HA, 2022).

The larger mature leaves area observed in T3 compared to T1, and T4 when compared to T2, during maximum stress conditions, could be result from the cell expansion, as treated plants might have experienced enhanced cell expansion due to nitrogen availability in the fertilizer, resulting in the synthesis of proteins and the production of enzymes and structural components essential for cell growth. The essential component of amino acids, which serve as the building blocks for proteins, is nitrogen (WAGNER, 2011). Additionally, increased leaf area witnessed in T1 compared to T3 during rehydration condition could be attributed to hormonal responses, because hormonal regulation plays a crucial role in plant growth and development,

including responses to water availability. The non-treated plants may have experienced more robust hormonal activation resulting in a larger increase in leaf size. Hormones such as auxins and gibberellins are known to stimulate cell division and elongation, contributing to increased leaf size (MORROW MELISSA HA, 2022).

Result of immature leaf area indicated larger leaf area in T4 compared to T2 under maximum stress conditions consequence of which might be related to cellular protection due to presence of nitrogen in the amino acid enriched fertilizer that resulted in nitrogen metabolism which helped in the synthesis of organic compounds that act as osmoprotectants and antioxidants that can help plants cope with drought stress by protecting cellular structures and minimizing damage caused by ROS allowing the plants to maintain healthy cell growth and development, leading to larger leaf area in the immature leaves. IQBAL et al., (2020), reported that by increasing nitrogen metabolism and antioxidant defense, fertilizers with high nitrogen can help plants cope with drought stress. The observed decrease in leaf area of immature leaves in T4 compared to T2, during rehydration could be attributed to delay recovery. While non-treated plants could quickly resume active growth and expansion upon rehydration, the treated ones might have required more time to readjust their physiological processes and resume normal growth.

The increased plant height observed in T3 compared to T1 during maximum stress condition, could be attributed to nutrient availability due to presence of nitrogen in the fertilizer. Nitrogen availability can enhance auxin biosynthesis and transport, leading to increased cell elongation and ultimately contributing to increased plant height. A study found that the auxin-homeostasis-maintaining gene dull N-response 1 (DNR1) can boost auxin biosynthesis and trigger auxin-responsive TFs OsARFs to activate OsNRT1.1B, OsNRT2.3a, and OsNPF2.4, boosting NO<sub>3</sub> uptake in roots (XING et al., 2023). The observed increase in T4 compared to T2 during rehydration might be due to enhanced recovery processes. Amino acid enriched fertilizer might have stimulated physiological processes involved in recovery from drought stress as nitrogen from the fertilizer could have facilitated the synthesis of proteins and enzymes such as amino acids involved in cellular repair and growth recovery (ABID et al., 2018). This improved recovery capacity could have contributed to increased cell elongation and consequently increased plant height.

The observed increase in diameter in T4 compared to T2 under maximum stress conditions, could be due to enhanced water uptake and transport. The application of organic fertilizer might have stimulated root growth and increased the absorptive surface area, allowing

the treated plants to access water more efficiently. This is because Improved water uptake and transport through the plant's vascular system can contribute to higher turgor pressure and cell expansion, resulting in increased stem diameter. ANAS et al., (2020), reported that nitrogen can stimulate root development, increase root volume, area, diameter, total root length, and dry mass, which will increase nutrient intake and improve nutritional balance as well as dry mass production. The decreased diameter observed in T3 compared to T1, under same condition, could be due to carbon allocation as nitrogen availability can influence carbon allocation within the plants. The plants might have allocated a larger proportion of carbon resources towards above-ground biomass rather than radial growth, as we witnessed increased in mature leaves of this plant during rehydration condition. Nitrogen addition, influence how plants allocate absorbed carbon, repair carbon during photosynthetic processes, and store carbon (WANG et al., 2019). The increase in diameter observed in treated plants during rehydration compared to non-treated plants could be attributed to enhanced nutrient availability and metabolism. The fertilizer application might have improved nutrient availability, including nitrogen. Enhancing physiological processes like photosynthesis, glucose metabolism, and protein synthesis can be accomplished by increasing the availability of nutrients. These actions support cell lengthening, expansion, and division, which together increase stem diameter in the treated plants (MU; CHEN, 2021).

## **7.CONCLUSION**

In conclusion, the key insights from this study have significant implications for sustainable agriculture and crop production, as foliar application of organic fertilizer effectively mitigates water deficit stress in common bean. This approach allows direct bypassing of water scarcity limitations on root uptake and nutrient assimilation, leading to enhanced nitrogen availability, improved physiological and metabolic processes in plants, and, ultimately, increased drought tolerance in common bean. Additionally, foliar application promoted nitrogen assimilation, protein synthesis, water-use efficiency, and overall plant performance, resulting in higher grain yields under water-deficit conditions.

Overall, the findings of this study underscore the potential of foliar application of organic fertilizer as a strategy to enhance water deficit tolerance and grain yield in common bean.

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