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Effects of Copper and Manganese on wheat and the involvement of the phytohormones, Methyl Jasmonate and Strigolactone on its stress tolerance

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Dedication

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Table of Contents

Abstract

70% of all heavy metals (HMs) and their compounds found in the human body come from food. HMs are natural trace elements with a density greater than 5 g cm^{-3} and could either be essential or non-essential. For example, copper (Cu), and manganese (Mn), are essential for the growth of plants in low concentrations, but their excessive amounts in the soil above threshold values result in toxicity. Due to an exponential increase in the use of HMs in several industrial, agricultural, domestic and technological applications, human exposure to HMs has increased. Thus, the importance of improving agricultural yield and remediating the environment cannot be overemphasized. Finding ways to enable agricultural plants to grow in suboptimal environmental conditions which will not only increase food production but remediate the environment is a hot topic of our time. Previous studies have established that exogenous application of phytohormones can positively influence key activities in plants, for example, the regulation of the ascorbate–glutathione cycle and cell division, hence improving plant growth activity. In this study, we investigated the involvement of phytohormones on the growth performance and stress tolerance of wheat. *Triticum aestivum* most widely cultivated wheat was used as an agricultural plant to investigate firstly, the effects of different concentrations of Cu and Mn on the plant's metabolic system. Cu and Mn are HMs that are currently widely used in many technical fields and agriculture. Secondly, to investigate the possible involvement of phytohormones Methyl jasmonate (JA) and Strigolactone (SL) on the growth performance of wheat, and thirdly, to investigate the possible involvement of phytohormones JA and SL on the ability of wheat plants to withstand heavy metal-induced stress. Methyl jasmonate is an important cellular regulator well reported to be involved in diverse developmental processes. Strigolactone is a small class of carotenoid-derived compounds important stimulants of germination in root parasitic plants and currently reported to play an important role in the architecture of plants under suboptimal environmental conditions. This study was carried out in a two-phased experimental design, in which the effects of HMs and the possible involvement of two phytohormones on the growth performance and stress tolerance of wheat plants was investigated. The results showed that, upon treatment of wheat plants with HMs, the negative effects on the plant's parameters studied increased with an increased concentration of the respective HMs applied to the soil. For the possible involvement of the phytohormones on the growth performance and stress tolerance of wheat, both phytohormones inconsistently influenced all plant parameters studied. Firstly, JA in all concentrations applied variably affected all parameters studied but generally speaking, JA 10^{-5} M and 10^{-6} M were the concentrations that frequently showed significant influences on the various parameters studied. The root length and FV/FM ratio were the plant parameters most frequently significantly influenced by the application of JA. Secondly, all concentrations of SL applied inconsistently affected all plant parameters studied. But generally speaking, the concentration 10^{-5} M most frequently showed significant effects on the plant parameters studied. The root weight was the parameter most frequently influenced by the application of SL. We, therefore concluded that the intensity of the effects of HMs Mn and Cu on wheat was dependent on the concentration of the HMs in the soil. Secondly, the application of the phytohormones JA and SL showed significant changes even though not consistent in the plant parameters measured and thus were implicated in the growth performance and the HM stress tolerance of wheat.

Zusammenfassung

70% aller im menschlichen Körper vorkommenden Schwermetalle (HMs) und ihrer Verbindungen stammen aus der Nahrung. HMs sind natürliche Spurenelemente mit einer Dichte von mehr als 5 g / cm^3 und können für Pflanzen, Tiere und Menschen essentielle Elemente sein. So sind beispielsweise Kupfer (Cu) und Mangan (Mn) für das Wachstum von Pflanzen in geringen Konzentrationen wesentlich, aber Mengen im Boden über den Schwellenwerten führen zu Toxizität. Aufgrund einer exponentiellen Zunahme der Verwendung von HMs in mehreren industriellen, landwirtschaftlichen, häuslichen und technologischen Anwendungen führt auch zu vermehrtem Kontakt des Menschen mit Schwermetallen. Daher kann die Bedeutung eines höheren landwirtschaftlichen Ertrags im Zusammenhang mit der Sanierung der Umwelt nicht genug betont werden. Es ist ein zentrales Thema unserer Zeit, Wege zu finden, wie landwirtschaftliche Pflanzen unter suboptimalen Umweltbedingungen wachsen können, und dabei nicht nur die Lebensmittelproduktion zu steigern, sondern auch die Umwelt zu schonen. Frühere Studien haben gezeigt, dass die exogene Anwendung von Phytohormonen Schlüsselaktivitäten in Pflanzen positiv beeinflussen können, beispielsweise die Regulation des Ascorbat-Glutathion-Zyklus und die Zellteilung, wodurch die Pflanzenwachstumsaktivität verbessert wird. In der vorliegenden Studie untersuchten wir die Beteiligung von Phytohormonen an der Wachstumsleistung und Stresstoleranz von Weizen. *Triticum aestivum*, der bekannteste und am weitesten verbreitete Weizen , wurde als landwirtschaftliche Pflanze verwendet, um zunächst die Auswirkungen verschiedener Konzentrationen von Cu und Mn auf das Stoffwechselsystem der Pflanze zu untersuchen. Cu und Mn sind HMs, die gegenwärtig in vielen technischen Gebieten und in der Landwirtschaft weit verbreitet sind. Zweitens, um die mögliche Beteiligung der Phytohormone Methyljasmonat (JA) und Strigolacton (SL) an der Wachstumsleistung von Weizen zu untersuchen, und drittens, um festzustellen, ob die beiden Phytohormone JA und SL die Fähigkeit Schwermetall-induziertem Stress zu widerstehen in Weizen beeinflussen. Methyljasmonat ist ein wichtiger zellulärer Regulator, von dem berichtet wird, dass er an verschiedenen Entwicklungsprozessen beteiligt ist. Strigolacton ist eine kleine Klasse von Carotinoiden-abgeleiteten Verbindungen, die wichtige Stimulanzien für die Keimung wurzelparasitärer Pflanzen darstellen. Derzeit wird außerdem davon ausgegangen, dass Strigolacton eine wichtige Rolle in der Architektur von Pflanzen unter suboptimalen Umweltbedingungen spielt. Diese Studie wurde in einem zweiphasigen Versuchsaufbau durchgeführt, in dem die Auswirkungen von HMs und die mögliche Beteiligung von zwei Phytohormonen auf die Wachstumsleistung und Stresstoleranz von Weizenpflanzen untersucht wurden. Die Ergebnisse zeigten, dass bei der Behandlung von Weizenpflanzen mit HMs die beobachteten negativen Auswirkungen auf die Pflanzenparameter mit einer erhöhten Konzentration der jeweiligen auf den Boden aufgebrachten HMs zunahmen. Für die mögliche Beteiligung der Phytohormone an der Wachstumsleistung und Stresstoleranz von Weizen beeinflussten beide Phytohormone inkonsistent alle untersuchten Pflanzenparameter. Erstens beeinflusste JA in allen angewendeten Konzentrationen unterschiedlich alle untersuchten Parameter, aber im Allgemeinen waren JA 10^{-5} M und 10^{-6} M die Konzentrationen, die am häufigsten signifikante Einflüsse auf die verschiedenen untersuchten Parameter zeigten. Die Wurzellänge und das FV / FM-Verhältnis waren die Pflanzenparameter, die durch die Anwendung von JA am häufigsten signifikant beeinflusst wurden. Zweitens wirkten sich alle SL-Konzentrationen inkonsistent auf alle untersuchten Pflanzenparameter aus. Jedoch zeigte die Konzentration 10^{-5} M im allgemeinen am häufigsten signifikante Auswirkungen auf die untersuchten Pflanzenparameter. Das Wurzelgewicht war der Parameter, der am häufigsten durch die Anwendung von SL beeinflusst wurde. Wir schließen daraus, dass die Intensität der Auswirkungen von Mn und Cu auf Weizen von der Konzentration von HM im Boden abhängt. Zweitens zeigte die Anwendung der Phytohormone JA und SL signifikante Veränderungen, auch wenn diese in den gemessenen Pflanzenparametern nicht konsistent waren und somit Einfluss auf die Wachstumsleistung und die HM-Stresstoleranz von Weizen hatten.

1. Introduction

In the last few decades, there has been an increasing awareness of the influence of heavy metals (HMs) as environmental pollutants. This is principally due to the fact that they can easily be assimilated into biological cycles (Baker and Walker, 1989). HMs are defined as metallic elements that are approximately five times heavier than water (Ferguson, 1990). They are naturally present in the soil, but geologic and anthropogenic activities increase the concentration of these elements to amounts that become harmful both to plants and animals.

Nowadays, there has been a growing ecological and global health concern linked with environmental contamination of HMs. This is basically as a result of the fact that the use of HMs, for example, in industrial and agricultural applications has increased the exposure of humans to toxic levels of these metals (Raskin *et al.*, 1994; Shen *et al.*, 2002). In that light, food consumption is now linked to 70 % of HMs and their compounds found in the human body (Jaishankar *et al.*, 2014). Therefore, in the nearest future HMs may become the most dangerous contaminants to human health, possibly surpassing solid and nuclear waste (Asgari Lajayer, Ghorbanpour and Nikabadi, 2017). Some key human practices which contribute to environmental pollution of HMs include mining, and the use of pesticides and fertilizers in agriculture (Raskin *et al.*, 1994; Shen *et al.*, 2002).

The desperate need to improve today's agriculture and its technological applications and food systems to meet up with the needs of a world population projected to increase to 9 billion by the middle of this century, cannot be overlooked (United Nations, 2017). Unfortunately, the ever-increasing demand for food, energy, land, and other natural resources, are still on the rise, placing ecosystems under increasing levels of stress. HM pollution in soil, poses a serious problem, linked to gross food production as a result of plant growth reduction, due to changes in physiological and biochemical processes in plants growing on such soils (Chatterjee and Chatterjee, 2000; Oancea, Foca and Airinei, 2005). Because plants are unable to build biomass, this affects the general crop yield. Thereby, directly affecting the security of food. Therefore, the identification and enhancement of plants to grow on HM soils and the remediation of HM polluted soils cannot be overemphasized.

Numerous methods of remediating metal-polluted soils exist; they array from physical and chemical methods to biological methods. For example, vapor extraction, stabilization, and soil washing are some of the examples of physical and chemical methods that are widely known. These methods are expensive and, in most cases where they have been applied, do not facilitate the establishment/reestablishment of plants on polluted soils. Biological methods (bioremediation) on the one hand are more advantageous to be used against the physical and chemical methods because they are not only cheap but do facilitate the establishment/reestablishment of plants on polluted soils. Furthermore, bioremediation techniques are considered to be environmentally friendly because, they are realized via natural processes (Sytar *et al.*, 2019). One such bioremediation technique, which involves the use of phytohormones has become a fascinating area of research of our times and is the main focus of this study.

It is important to note that, HMs play an important role in plant growth and development as micronutrients. However, above threshold values, these HMs act as toxins and cause stress on the metabolism of plants leading to growth reduction. Thus, finding eco-friendly, sustainable and economical methods to tackle this problem is very trivial. The ascorbate–glutathione cycle and transpiration rate are some of the core activities in plants that have been shown to be positively influenced by the exogenous application of phytohormones, thus greatly improving plant growth, yield and stress resistance (Sytar *et al.*, 2019). Current research suggests that plants are primed by phytohormones for stress tolerance due to the crosstalks with exogenously applied phytohormones. Chemical priming has delivered good outcomes in plant physiology and stress adaptation, and phytohormone priming is in progress.

In this study, it was purported that different concentrations of Cu and Mn, would induce varying levels of stress on wheat plants and that the application of different concentrations of phytohormones JA and SL, may increase the performance of wheat plants and also be involved in the stress tolerance of wheat to HMs, Cu, and Mn. Plants were grown in two badges on soil substrate at graded concentrations of HMs, Cu, and Mn. The first badge of plants was grown for a total of five weeks and treated with MeJA. The second badge of plants for four weeks and treated with GR24. Within the time frame of each experiment, the level of chlorophyll fluorescence was measured, expressed as a ratio of the variable fluorescence to the maximum fluorescence (FV/FM ratio), and the cellular tolerance by the method of plasmolysis was also measured. At the end of each experiment, the plants were harvested and plant

growth parameters such as the root and shoot length, root and shoot weight were measured, and the number of leaves counted.

1.1. Heavy Metals (HMs)

HMs are natural trace elements, with an average concentration in the earth 's crust of < 0,1% (1000 g per Ton), density greater than 5 g cm^{-3} and could either be essential or non-essential (Boryło, Nowicki and Skwarzec, 2013). For example, copper (Cu), and manganese (Mn), are critical for the growth of plants in low concentrations, but their excessive amounts in the soil above threshold values can result in toxicity (Ashraf, Ahmad and Ozturk, 2010). The type and amount of HM in the soil are dependent on the history of the HM place. Based on syntaxonomy, three types of HM vegetations can be distinguished which include: primary, secondary and tertiary sites. Due to phytotoxicity on metalliferous soils, these sites are very restrictive to the general population of plants. Thus, allowing only specific plant populations which can develop specific coping mechanisms at these island sites. This brings about selective pressures within plant communities such that the species that mostly inhabit HM sites are genetically altered. These ecotypes usually comprise of a group of plants which show specific tolerance to specific HMs at the specific sites. This adaptation is developed by the plants via microevolutionary processes at the specific site (Ernst, 2006). These plant species with a unique characteristic to survive on HM soils are referred to as metallophytes. Metallophytes could either be; (a) Obligate metallophytes: and (b) Facultative metallophytes (Ashraf, Ahmad and Ozturk, 2010). On the other hand, non-metallophytes are the group of plant species that are incapable to survive on metalliferous soils, for example in our case wheat. Generally, most plant species fall within this category.

1.1a. Bioavailability and uptake

In soil, the solubility and bioavailability of HMs to the plant's roots are dependent on a number of chemical properties of the soil such as the soil pH and organic matter content (Williams, Vlamis, Purkite, 1980; Logan, 1983). Generally, the higher the organic matter and soil pH, the metals will have a longer residence time in the soil because they will be strongly bound to the soil particles and will be less bioavailable to plants. Plants as well, influence the bioavailability of metals through several means, for example, exudation of carboxylates. The most common exudate by plants is the secretion of phytosiderophores into the rhizosphere to chelate and solubilize metals that are soil-bound (Kinnersley, 1993). This complex formation augments metal solubility and consequently provides a better uptake into the plant. Additionally caffeic acid from Arachis hypogaea (Römheld and Marschner, 1986), flavonoids from Lupinus albus (Weisskopf *et al.*, 2006), and flavins from Beta vulgaris (Cesco *et al.*, 2010) are some of the well-known phenolic compounds released by plants upon iron deficiency. After HMs have been released from the soil particles and are available in the soil solution, they are taken up by root cells of the plants in the soil. The movement of these metals into the plant from the soil is mainly dependent on, the probability of the roots getting in contact with the HMs, the flow of the HMs from the soil down the water potential gradient and diffusion of the metal elements into the plant (Marschner, 1995b).

However, reports show that a 'Soil–Plant Barrier' may well protect the food chain from toxicity of HMs which implies that, levels of HMs in edible plant tissues are reduced to levels safe for animals and humans by either, prevention of uptake of metal elements due to their insolubility in soil, prevention of translocation of metal elements by making them immobile in roots or lowering the phytotoxicity of the metal elements to permissible level both for animals and human beings (Brandt and Hendrickson, 1990). To attain the aforementioned soil-plant barrier, plants have developed a series of mechanisms to avoid HM toxicity which includes: the blocking of main functional groups, reactive oxygen species production and plant's ability to displace metal ions from biological molecules (Clemens, 2006). The cell wall has a comparatively low affinity and low selectivity whereby metals are first bound. For the metals to be taken up from the cell wall into the plasma membrane, the transport system and the high affinity intracellular binding sites coordinate and drive this uptake activity. A very important characteristic of the plasma membrane which facilitates the uptake of these metals through secondary transporters is the fact that the membrane is negative on the inside and may exceed – 200mV in root epidermal cells (Hirsch *et al.*, 1998). However, for example, HMs uptake by roots can either be passive or partially passive (Cataldo *et al.*, 2019).

In the case where HM elements enter the cytoplasm, especially for adapted tolerant plants, they are bound immediately by an appropriate cellular compound. This helps to reduce any toxic effects that can be brought about by these free cellular metal ions on the plant's metabolic system. And also, for example, provide a more constructive involvement of these metals in specific metalloproteins. The reduction of the toxicity of HMs in the cellular space is referred to as HM chelation and well-known cellular metal chelators are nicotianamine (NA) and organic acids like citrate (Rauser, 1999; Curie *et al.*, 2009). With an increasing metal concentration in the external medium, the uptake of metals, both by roots and leaves increases. Nevertheless, the uptake has no linear relationship with increasing concentration. Depending on the competition of HMs at the uptake sites, saturation can result wherein the uptake of the HMs equalizes with the HM content of the tissue. Once the metal has been taken up by the root symplasm movement to the shoots through the xylem depends on, metal sequestration into the root symplasm, symplastic transport into the stele, and release of metals into the xylem (Ashraf, Ahmad and Ozturk, 2010). The ion transport into the xylem is generally mediated by membrane transport proteins. Nevertheless, the shoot/root concentration ratios are highly dependent on the plant species, whereby some have really high ratios while others very low concentration ratios (Zayed and Terry, 2003b).

1.1b. Accumulation and sequestration

A greater proportion of the plant kingdom belongs to the group of non-accumulator plants for example, wheat, which in respect of this study is the experimental plant of choice. Nevertheless, plants generally have to put up with even low or high levels of HMs in the soil for nutrition purposes and even growth. Therefore it is very trivial for plants to possess finely tuned mechanisms to enable them to establish and grow on soils with even toxic levels of HMs (Hall, 2002; Clemens, 2006). These mechanisms have led to the division of plants into various subgroups based on their level of accumulation and exclusion of HMs in their below ground or above-ground tissue. Plants are grouped into three main subgroups depending on their behavior on HM soil which could either be; metal indication, metal chelation, and metal hyperaccumulation (Baker, Reeves and Hajar, 1994; Raskin *et al.*, 1994). However, the unique importance of metal hyperaccumulation in plants is still very elusive but it has been suggested to be a special trait in plants that offers defense against pathogen and/or pathogen attack (Freeman *et al.*, 2006; Boyd, 2007). This knowledge has broad relevance in general for the study of the accumulation of toxic metals or developments of lack of essential micronutrients throughout the food chain (the idea of biofortification) and for phytoremediation or Phyto mining processes.

Generally, the level of tolerance showed by plants to HMs is dependent on sequestration and efflux which are considered the key processes of basal tolerance (Clemens, 2006). These processes lead to metal chelation and compartmentalization. Metal sequestration in discrete cellular compartments plays a fundamental role in metal tolerance and supplement with essential metals. A good example is the insertion of metal-phytochelatine complexes into the vacuole (Schneider *et al.*, 2009). One important mechanism used by plants to withstand high concentrations of HMs in the soil is the production of increased amounts of hormones for example hormones such as abscisic acid or ethylene have been shown to increase in plants during HM exposure (Schneider *et al.*, 2009; Maksymiec, 2011).

For a clear understanding of these hormonal involvements, transcriptomic studies have been carried out and have revealed that metal hyperaccumulation has a lot to do with specific genes. For example in *Arabidopsis halleri* hyperaccumulation was shown to be associated with more than 30 candidate genes which were highly expressed compared with the nonaccumulator *Arabidopsis thaliana* (Becher *et al.*, 2004). Pence *et al.*, (2000), could show that the hyperaccumulator *Noccaea caerulescens* and the non-accumulator *Noccaea* arvense are both different in that they both express the gene ZnTI, which is a Zn^{2+} transporter differently. He showed that the hyperaccumulator *Noccaea caerulescens* expressed the gene more than the *non*-accumulator *Noccaea arvense* (Papoyan and Kochian, 2004) which might give reason to the fact that the latter is an accumulator.

1.1c. HM homeostasis

Due to the fact that HMs interact with the cellular redox environment in different ways, the tight regulation of metal homeostasis in plants is a widespread network consisting of various elements. One which is of great importance is the generation of reactive oxygen species (ROS). ROS is directly

generated through Fenton like reactions and the Haber-Weiss cycle by Redox-active metals (Stohs and Bagchi, 1995; Sharma and Dietz, 2009). For the detoxification of these toxic hydroxyl groups generated by HMs in the cell, a major element of cellular redox homeostasis called glutathione (GSH) either acts as a direct chelator or precursor of phytochelatines. The detoxification of ROS is GSH dependent; GSH either directly or indirectly removes ROS generated by metals. In the plant cells, excess levels of ROS induce GSH synthesis which is supposed to only be moderately destabilized otherwise plants get stressed (Noctor *et al.*, 2011).

Generally, plant cells can withstand a slight imbalance in the levels of GSH in the cell, however, elevated levels of HM destabilize the equilibrium of this GSH pool thus leading to hypersensitive responses by plants cell. Another GSH consuming process that contributes to the glutathione homeostasis is the synthesis of phytochelatines (PC) (Grill, Winnacker and Zenk, 1985). Even though antioxidant defense processes try to keep the ROS produced in response to the HM toxicity low, nevertheless, this equilibrium is still disrupted for example, in the case where there is an increased concentration of the HM, or low tolerance of the specific plant species (Sharma and Dietz, 2009). Plant cells bear a sophisticated network of antioxidants that are composed of non-enzymatic molecules such as GSH, superoxide dismutase (SOD), glutathione reductase (GR) and catalases. These molecules form the bases of a plant species exhibiting a strong antioxidant defense system in HM tolerance. However, there is no dependable basis for defining mechanistic relationships, due to the lack of unique patterns of enzyme activity. Therefore, the HM, its concentration and the plant species should be carefully considered when investigating redox imbalances and oxidative stress induced by HMs.

HMs are inherently toxic in that they generate signals/symptoms by interacting with plant metabolic pathways. These symptoms/signals that are generated in plants by metal toxicity have been studied in a number of plants exposed to a great number of different environmental conditions. They can be divided into direct targets, which are often metalloproteins and metal-binding molecules e.g. chlorophyll or indirect metal-induced impairments of physiological pathways (Küpper, Küpper and Spiller, 1996). Metalloproteins encompass coordinated transition metals which can be replaced by chemically analogous other transition metals. Some indirect metal-induced impairments of physiological pathways here include, growth rate (e.g. Shanker *et al.*, 2005), germination rate and establishment ability (e.g. Seregin and Ivanov, 2001), root morphology (e.g. Salt *et al.*, 1995) stem morphology (e.g. Rout, Samantaray and Das, 1997), leaves morphology (e.g. Shanker *et al.*, 2005), biomass (e.g. Tokalioğlu and Kartal, 2006), and water uptake (Kramer and Boyer, 1995).

1.2. Copper

Cu is a metal that is reddish in color and occurs naturally or can be anthropogenically influenced in the environment. Naturally, Cu can be found in rock, water and sometimes at very low concentrations in air. Its normal concentration in the earth's crust is around 50 parts copper per million parts soil (ppm). Anthropogenically, Cu concentrations are increased in the environment due to human influences of which principally involves the use of Cu in several human technical fields as raw materials and in agriculture as well (Vlcek and Pohanka, 2018). Deficiency of this element can occur in peat and sandy soils as well as in intensively cultivated soils of other types due to improper fertilization and an unfavorable Cu: N balance (Bussler and Rahimi, 1973) In soil solution Cu ions are relatively always very low in concentration, Due to the fact that Cu is known to bind very strongly to soil particles, clay minerals (Al-Qunaibit, Mekhemer and Zaghloul, 2004), organic matter (Cavallaro and McBride, 1984; Boujelben, Bouzid and Elouear, 2009), etc. Cu has a very high affinity for organic matter and the bond between organic matter and Cu is much stronger than with other HMs (Adriano, 2013).

Cu is a very essential element for both plants and animals. Its redox-active characteristic makes it an important participant in many physiological processes in plants and animals (Yrule, 2005). Cu can be; a structural element involved in certain metalloproteins, a co-factor in enzymes, plays a vital role in the metabolism, signaling and transduction of cell wall etc. (Puig *et al.*, 2007). With the aforementioned roles played by Cu in plants, it is therefore logical to state that plants require Cu for normal growth and development. On the other hand, some particular deficiency symptoms are developed by plants when there is a deficiency of Cu. These deficiencies are first noticeable in young leaves and reproductive organs.

However, the redox properties that make Cu an essential element for plants also contribute to its inherent toxicity. When plants are exposed to Cu above threshold values, this can lead to the destruction of cells

at the level of lipids, membranes, nucleic acids and proteins. This is brought about by the redox cycling between Cu^{2+} and Cu^{+} which catalyzes the generation of highly toxic hydroxyl radicals (Halliwell and Gutteridge, 1984). Even though Cu is very important in plant cells and mostly binds to proteins, its inherent ability to cause oxidative damage can lead to the interference of important processes in the plants cell such as photosynthesis and other metabolic mechanisms thereby giving rise to strong retardation in the growth rate of plants (Van Assche and Clijsters, 1990; Yrule, 2005). Cu above threshold values can bring about (physically observable) impediments in plant growth parameters such as chlorosis, necrosis, and stunting. In plant cells, the binding of proteins to excess Cu is unavoidable. When this happens a number of protein characteristics are lost for example the binding of Cu to sulfhydryl groups in proteins can lead to the loss of enzyme activity or functions, secondly can lead to a deficiency in other essential ions, thirdly can lead to the impairment of cellular transport processes and fourthly oxidative stress. Nevertheless, plants show the same physiological symptoms upon Cu deficiency or excess (Yrule, 2005).

To avoid any kind of toxicity brought about by the redox cycling of Cu in the plant cell, the acquisition of Cu from uptake by roots from the soil through the plant to the point of distribution and compartmentalization in the respective tissues must be regularized and well structured. Importantly the concentration must be regularized within different cells and organelles within a very narrow physiological range. Therefore, plants have the unique ability to acquire the right amount of Cu from diverse environmental conditions and delivering this element to specific plant compartments and targets (metalloproteins), while avoiding the toxic effects that can be brought about by transporting these elements within the confines of their vessels. Therefore in plants, there is a complex and regulated interacting network that controls the acquisition and assimilation of Cu which is principally dependent on plant mineral supply and demand (Marschner, 1995a). Cu homeostasis processes generally act in response to the availability of metals, different annual cycles, and the different growth stages of a plant, these three are dynamic in nature.

The concentration of Cu in plants vegetative tissues varies significantly depending on the plant species, developmental stage and environmental factors. Environmental factors such as the concentration of nitrogen in the soil and the chemical properties of the soil also contribute to the amount of free Cu in soil solution. For example, soils with very high concentrations of Nitrogen have been shown to significantly need more Cu supply. Furthermore, Cu availability in soil solutions tends to increase as the soil pH decreases. The concentration of Cu in plant tissue varies between low "1" and high "5" mg g^{-1} dry weight (e.g. Marschner, 1995), and in leaves ranges between low "5" to high "20" mg g^{-1} dry weight and averagely in leaves 10 mg g^{-1}. Even though these concentrations can vary between different plant species and tissues, generally in all plant cells free Cu concentrations are bound to be kept at very low concentrations due to its high redox properties. In the nutrient media Cu concentrations usually ranges from 10^{-14} to 10^{-16} M above and below which there is an excess or deficiency respectively. In the soil Cu concentrations usually range from 10^{-6} to 10^{-9} M (Marschner, 1995a). Concentrations of free metal ions in the soil solution are always kept on a low margin, although this depends on soil type and chemical properties (Mortvedt *et al.*, 1991).

Cu characteristically by the method of complexation or absorption associates with inorganic and organic matter in both soil solution and solid phase. Its ions show a very strong affinity to binding sites of soil components, surfaces of clay as well as Mn and Fe oxides etc. Cu also binds to cell walls and also on the surface of plant roots cells. Depending on how tightly these Cu ions bind on these plant surfaces determines the chemical mobility and hence the amount of Cu that will finally be taken up by the plant. In the soil, the pH significantly influences the number of free Cu ions in solution. The lower the pH value, competitive adsorption arises between organic matters in the solid phase and dissolved organic carbon. This gives rise to an increase in Cu ions in soil solution due to the fact that there is an increase in the total amount of dissolved organic carbon and vice versa (Carrillo-González *et al.*, 2006). Thus, upon increasing pH, the Cu ions activity will considerably decrease at the expense of organically bound complexes in the soil solution (Sauvé *et al.*, 1997). Additionally, the activities of roots and microbes in the rhizosphere can as well influence the soil pH and or dissolved organic carbon content of the soil, thereby, influencing the chemical mobility and consequently release and uptake of metal ions (Hinsinger and Courchesne, 2008).

Once Cu has been taken up into the plant cell and appropriately stored, It is required in at least six locations, for example, the chloroplast stroma and cytosol, just to name a few (Marschner, 1995a). Until very recently the acquisition and transport of Cu within cells was very unclear. Nevertheless, recent applications of knowledge gained from the understanding of yeast cells on other eukaryotic cells has brought illumination to this area. Consequently, several families of HM transporters involved in

intracellular homeostasis have been identified in plants. For example, Cu transporter protein (COPT) (Puig *et al.*, 2007), P-type HM ATPases (Solioz and Vulpe, 1996), and metallochaperones (O'Halloran and Culotta, 2000). The just mentioned Cu transporters are required by plants as specialized proteins preventing and limiting the reactivity of Cu^{+} ions inside the plant cell. These Cu ions have been well documented to change a series of gene expressions leading to morphological and physiological changes in plant roots and/or leaves.

Cu is toxic to almost all plant species, but for a few that have the ability to hyper accumulate this high redox cycling metal element. It is important to keep in mind that when studying the effects of Cu on plants, the plant species, the concentration of Cu supplied, exposure time and soil properties should be considered (e.g. Strzalka, 1999).

1.3. Manganese

Manganese (Mn), is a transitional element, silver-grey in color and ferrous. It is a very important metal in modern-day industrial economies given that it is consumed in large quantities by industrialized countries (William and Kimball, 2017). It is the 12^{th} most abundant element and 5^{th} most abundant metal on earth. Mn is very easy to oxidize. The behavior of Mn in soils and plants reflects closely some important aspects of its chemistry. These include: firstly its stability at different concentrations e.g. Mn^{2+} is very stable in acid solutions and $Mn0_2$ is very stable in alkaline solutions in the presence of oxygen; secondly, its inability to form strong ligands, that is Mn^{2+} does not form complexes as strongly with inorganic or organic ligands as other micronutrients (Cu^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}); thirdly, its ability to function both as a Lewis acid and as an oxidation catalyst. In soil, oxides of Mn (III) (IV) are generally found in alkaline or oxidizing environments, whereas soluble and exchangeable Mn^{2+} are dominant under acid or reducing conditions (Graham and Hannam, 1988).

Mn concentrations in soil solutions can vary along a wide range which could be from 10^{-9} M to $l0^{-3}$ M with a majority of soil types with concentrations of Mn falling within the range of 10^{-7} M to $l0^{-5}$ M. Most of the Mn in soil solutions is present as the hydrated divalent ion although in neutral and alkaline soils soluble Mn silicates and bicarbonates may become significant species. Because of the similarity in binding characteristics of Mn with Ca and Mg, extensive complexing between Mn and organic ligands, including humic substances, is unlikely (Graham and Hannam, 1988). Mn is generally not found as a free element in the environment but usually exist as Mn oxides, Mn carbonates, and Mn silicates.

One of the important sources of free Mn in the environment is natural soil erosion which releases tons of Mn into the air, soil, and waterways on an annual basis. The release of Mn from bedrock and exposure by erosion makes this element readily available for microorganisms, plants and animals who later absorb it into their systems. Mn occurs ubiquitously in soil and generally in solid form but depending on the pH of the soil Mn can easily be solubilized especially at acidic pH values. Human activities such as mining contribute significantly to the release of toxic levels of Mn into the environment. With increased concentrations of Mn in the environment plants and animals are likely to suffer from Mn toxicity if proper control is not carried out (Yokel, 2009). Mn has unique physical and chemical properties that contribute to its significant importance in industrial settings for example in the manufacture of batteries, steel leather glass etc. It is also used in energy consumption, as well as in agriculture incorporated in pesticides and fungicides (Millaleo *et al.*, 2010).

The concentration of Mn in drinking water usually ranges from 1mg/L up to 2 mg/L depending on the distance of the water source from a Mn contaminated site (Frisbie *et al.*, 2002). Rice, nuts, whole grains and legumes contain the highest levels of Mn in human daily diets. leafy green vegetables, tea, chocolate, and seafood are also abundant in Mn (Mukhopadhyay and Sharma, 1991). Mn levels in the plant's metabolic processes could either be toxic or limiting. It functions as an essential micronutrient in the right amount but could lead to phytotoxicity when in excess (Kochian, Hoekenga and Piñeros, 2004; Ducic and Polle, 2005). Mn toxicity is favored in acidic soils. With decreasing pH, the amount of exchangeable manganese – mainly Mn^{2+} form – increases in the soil solution. This loose Mn form is readily available for plant roots which are easily transported into the root cells and translocated to the shoots system where it is finally accumulated into plant tissues (Marschner, 1995a). Mn (III) and Mn (IV) are some other forms of Mn that are abundant in soil solution at higher pH levels but these two are not readily taken up by plants because they cannot be accumulated by them (Rengel, 2000).

When the concentration of Mn within plant tissues increases above threshold values, this can bring about oxidative stress as well as alter important processes in the plant cells such as enzyme activity and

translocation. Additionally, this increase Mn levels can hinder the ability of the plants to take up and utilize other important mineral elements such as Ca, Mg and Fe (Ducic and Polle, 2005; Lei, Korpelainen and Li, 2007). The beginning of Mn damage and also the magnitude of tolerance exhibited by plants is mainly reliant on the plant species and genotypes within a species (Foy, Scott and Fisher, 1988). Because Mn plays a vital role in photosynthesis by participating as a structural element of photosynthetic proteins and enzymes, low Mn levels are absolutely necessary for normal nutrition and development of plants. Acting as an essential micronutrient, its deficit in plants can bring about defects in the chloroplast. Which can mainly be due to the fact that it affects the water-splitting system of photosystem II (PSII), which is the key supplier of the electrons necessary for photosynthesis (Buchanan, Gruissem and Jones, 2000).

Normal Mn contents of leaves fluctuate greatly between species 30-500 mg kg^{-1} Mn dry mass (Clarkson, 1988). Nonetheless, when it is present in excess it is extremely toxic to plant cells (Migocka and Klobus, 2007). Important symptoms of Mn toxicity in plants include, decrease in growth rate, chlorosis, and necrotic leave spots. These symptoms have been experimented and reported in plants like canola (Moroni, Scott and Wratten, 2003), clover (Rosas, Rengel and Mora, 2007), ryegrass (e.g Mora *et al.*, 2009) as well as in barley and cowpea (Demirevska-Kepova *et al.*, 2004). Necrotic brown spots and chlorotic leaves are frequently noticeable indicators of the severity of Mn toxicity in plants (Wissemeier and Horst, 1991). The chlorotic leaves of plants suffering from Mn toxicity look like those of plants with an Fe deficiency (Sarkar *et al.*, 2004). Furthermore, depending on the absence or low supply of other essential elements such as Ca, Mg, K, Fe, and Si, the intensity of Mn toxicity is intensified (Abou *et al.*, 2002). Nevertheless, plants have been reported to show a decrease in productivity with an absence of visible symptoms of toxicity on the leaves (Miner and Sims, 1983). It is important to note that Mn toxicity in plants directly affects the chloroplast bringing about defects in the photosynthetic apparatus and as such hindering the photosynthetic ability of plants giving rise to the observable characteristics.

1.4. Phytohormones

Phytohormones are essential chemical ingredients needed by plants to integrate endogenous developmental cues with environmental signals to regulate plant growth, development, and defense (Wasternack and Hause, 2002; Creelman and Rao, 2019). Just like every other living thing, plants have a life span, they are born, grow and die. Within this time, they produce these essential chemical ingredients to regulate their growth and development. Phytohormones are produced by plants at very low concentrations and at these low concentrations, they are effective. These chemical ingredients carry out their functions at the site where they are produced, or they could be carried to other tissues or parts of the plants where they are effective (Öktüren and Sönmez, 2005). In the past just five hormones where considered but in recent times hormones such as brassinosteroids (BR), salicylic acid (SA), Strigolactone (SL), and jasmonic acid (JA) have been considered to be phytohormones acting in the same line as, auxin, cytokinin, gibberellin, abscisic acid, and ethylene. Phytohormones, play a very vital role in the plant`s life cycle and these roles can be subdivided into three general categories which include; (i) hormones providing control of vegetative development, (ii) hormones controlling reproduction, and, (iii) hormones responding to stress (Mehmet. and Mustafa, 2018).

1.4a. Jasmonates (JAs)

Methyl jasmonate (MeJA) and jasmonic acid (JA) are collectively referred to as jasmonates (JAs). Jasmonates regulate very important cellular developmental processes such as seed germination, fruit ripening, etc. (Wasternack and Hause, 2002; Creelman and Rao, 2019). Numerous questions have arisen, based on the pleiotropic effects of JAs, on their ability to regulate biogenesis. Since the 1980s a great deal of information has been uncovered based on JAs biosynthesis and signaling pathway, as well as the crosstalk of JAs with other phytohormones during plant growth and development. Just like other renowned phytohormones, JAs integrate endogenous developmental cues with environmental signals to regulate plant growth, development, and defense. JAs play key defense roles in plants like activating the defense system of the plant to respond to biotic stressors such as insect-driven wounding, pathogens as well as abiotic stressors such as drought, low temperature, and salinity.

1.4a.1. Structure

JA is a monocarboxylic acid. It presents its self structurally as an acetic acid (3-oxocyclopentyl) that is replaced by a (2*Z*)-pent-2-en-1-yl group at the second position of the cyclopentane ring (Figure 1.1) (Hyun and Lee, 2008). JAs generally have a chemical formula of $C_{12}H_{18}O_3$ and an average Molar Mass of 210.27 M. Their boiling point is usually 160 °C or thereabout and they have a density of 1.10 g/cubic cm (Demole, Lederer and Mercier, 1962).

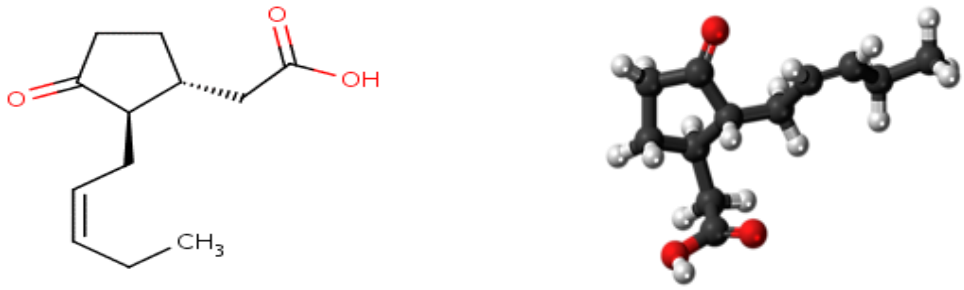


Figure 1.1: the general structure of Jasmonates (JAs). (1R,2R)-3-Oxo-2-(2Z)-2-pentenyl-cyclopentaneacetic acid (Andolfi *et al.*, 2014)**.**

1.4a.2. Occurrence

But for some prokaryotes, some lower and all higher plant species that have been shown to contain JAs, JAs, and components of their biosynthesis and signaling pathway, respectively, do not occur in yeast, in animal and human tissues (e.g Nishiyama *et al.*, 2018). The first detected JAs compound was the methyl ester of JA (JA-Me) found in the odor of flowering plant`s acid (Demole, Lederer and Mercier, 1962) and in the culture medium of the fungus *Lasiodiplodia theobromae* (synonym: *Botryodiplodia theobromae*) (Jesus *et al.*, 1987). Later on, specific stereo-isomeric forms of JAs such as (+)-7-iso-JAs and its derivatives were detected in *L. theobromae* (Miersch *et al.*, 1989). Lasiojasmonates which are referred to as JAs esters were isolated from different Lasiodiplodia species (Andolfi *et al.*, 2014). In the grapevine pathogen *Lasiodiplodia mediterannea* sp. The JA furanoyl ester LasA was discovered that it

could be transformed into the bioactive JA-Ile and seemed to function as an inactive JA pool (Chini *et al.*, 2018). Continuous improvement of analytical tools is expected to shed more light on the accuracy of some of the preliminary results available for lower organisms. In higher land plants, JAs compounds occur ubiquitously even the conjugate of (9S,13S)-12-oxo-phytodienoic acid (OPDA) with isoleucine has been found in flowering *Arabidopsis thaliana* and showed biological activity (Arnold *et al.*, 2016).

1.4a.3. Synthesis and perception

Using in vitro enzymatic methods, it has been recently clarified that, JAs are formed from the isoleucine (Ile) conjugate of α–linolenic acid (α-LeA) (Uchiyama *et al.*, 2018). Jasmonic acid (JA) and its isoleucine conjugate (JA-Ile) originate from lipids of chloroplast membranes, preferentially α-LeA. They are synthesized from α-LeA/18:3 via the octadecanoid pathway (e.g. Browse, 2009). The sequential conversion of, α-LeA produced via the synchronized actions of fatty acid desaturase (FAD) and phospholipase A1 (PLA) in plastids to (13S)-hydro peroxy octadecatrienoic acid (13-HPOT), 12,13(S)-epoxy octadecatrienoic acid (12,13-EOT), and OPDA is coordinated through the actions of 13-lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC). The produced OPDA is then transported to peroxisomes. In the peroxisomes OPDA is reduced by OPDA reductase (OPR) to 3-oxo-2-(cis-2′-pentenyl) cyclopentane-1-octanoic acid (OPC-8:0). The activation of OPC-8:0 by OPC-8:0 CoA ligase (OPCL) is carried out prior to the sequential shortening to Jasmonic acid. This step is made possible by three consecutive rounds of β-oxidation catalyzed by three different enzymes namely, acyl-CoA oxidase (ACX), multifunctional protein (MFP), and 3-ketoacyl-CoA thiolase (KAT). Jasmonic acid is then exported to the cytoplasm, where it is conjugated with isoleucine to form bioactive (+)-7-iso-JA-Ile, which can be inactivated to 12-hydroxy-JA-Ile by CYP94B3, a cytochrome P450, or metabolized to other inactive forms via methylation, glucosylation, or sulfation (Wasternack and Strnad, 2016). In the absence of JA, JA ZIM-DOMAIN (JAZ) proteins recruit NOVEL INTERACTOR OF JAZ (NINJA; an adaptor protein) and TOPLESS (TPL; a co-repressor) to repress various downstream transcription factors (TFs) via direct protein interactions (Chini *et al.*, 2007). Following the perception of bioactive JAs, the JAs receptor CORONATINE INSENSITIVE1 (COI1) (part of the SKP1/CULLIN-based SCFCOI1 E3 ligase) (Yan *et al.*, 2007) mediates the ubiquitination and degradation of JAZ proteins via the 26S proteasome. The resulting activation of TFs enables the expression of JA-responsive genes and JA responses (Huang *et al.*, 2017) (Figure 1.2).

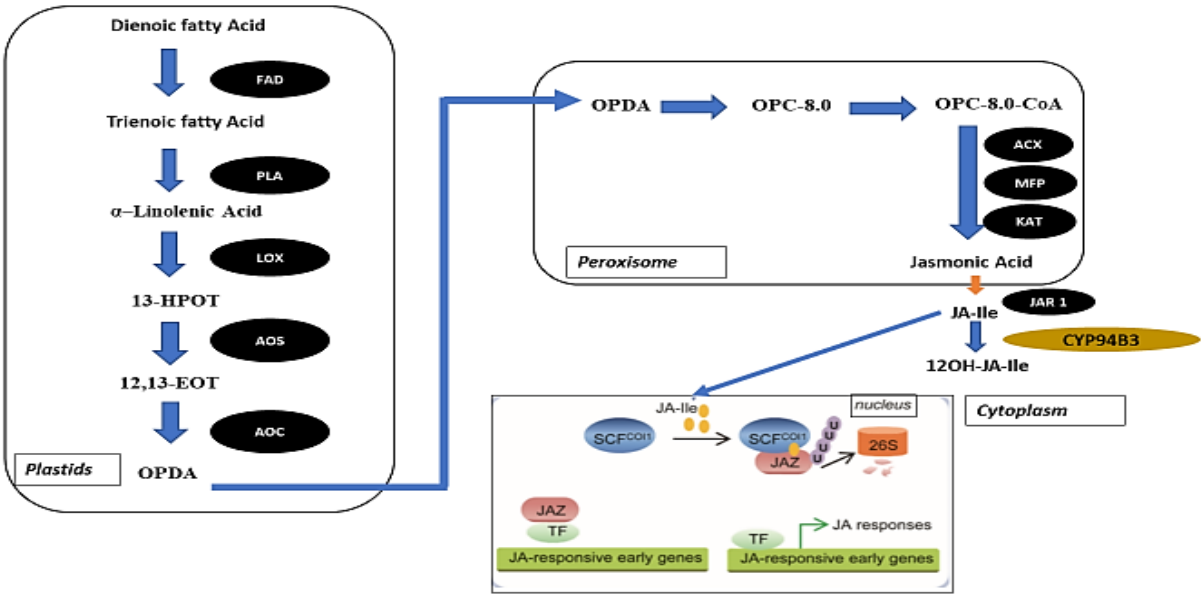


Figure 1.2: A summarized report of jasmonate (JA) biosynthesis and signaling. *ACX, acyl-CoA oxidase; AOC, allene oxide cyclase; AOS, allene oxide synthase; 12,13EOT, 12,13(S)-epoxyoctadecatrienoic acid; FAD, fatty acid desaturase; 13-HPOT, 13-hydroperoxyoctadecatrienoic acid; JA-Ile, jasmonoylL-isoleucine; 12OH-JA-Ile, 12-hydroxy-JA-Ile; JAR1, JASMONATE RESISTANT 1/jasmonate-amido synthetase; KAT, 3-ketoacyl-CoA thiolase; LOX, 13-lipoxygenase; MFP, multifunctional protein; PLA, phospholipase A1; OPC-8:0, 3-oxo-2(cis-2′-pentenyl)-cyclopentane-1-octanoic acid; OPCL, OPC-8:0 CoA ligase; OPDA, (9S,13S)-12-oxo-phytodienoic acid; OPR, OPDA reductase))*

1.4a.4. Crosstalk in the JA/GA-mediated repression of plant growth

The inhibitory effect of JAs on growth enhances survival in natural environments by allowing plants to concentrate on defending themselves against various stresses. In Arabidopsis, JAs inhibit growth and promote defense, whereas gibberellin (GA) acts antagonistically to the JAs response. GA acts

antagonistically by inducing the degradation of DELLA proteins, thereby removing JAs repressive effects on TFs known as PHYTOCHROME INTERACTING FACTORs (PIFs) and promoting plant growth. GA also allows JAZ to inhibit MYC2 and JA-mediated plant defenses. Conversely, JAs promote JAZ degradation to activate MYC2 for the enhancement of plant defenses and derepress DELLA proteins to inhibit PIFs, suppressing growth (Hou *et al.*, 2010). Some examples of previous studies on JAs involvement in plants include regulation of embryo/seed development (Goetz *et al.*, 2012), inhibition of petal expansion in Arabidopsis (Brioudes *et al.*, 2009; Reeves *et al.*, 2012), induction of leaf senescence (Qi *et al.*, 2015) inhibition of apical hook formation in Arabidopsis (Song *et al.*, 2014) and Delay of flowering in Arabidopsis (Zhai *et al.*, 2015).

1.4a.5. Application

JAs application has been shown to activate several plant defense mechanisms. For example, JAs application has been shown to delay the ABA-mediated inhibition of seed germination in Arabidopsis (Ellis and Turner, 2002). It also promotes trichome formation following wounding or herbivory (insect attacks). Trichomes are epidermal cell structures on the aerial parts of plants, they protect plants from herbivore attack by acting as sensors or barriers, or by releasing volatile compounds. Deficiencies in JA biosynthesis and perception in plants for example as studied in Arabidopsis have been shown to block wound inducement of trichomes (Yoshida *et al.*, 2009). Furthermore, JAs have been shown to be implicated in the regulation of stomatal closure and reopening in Arabidopsis. Stomata, leaf epidermal pores bordered by pairs of guard cells, regulate water loss, gas exchange, and thus plant immunity to pathogens and photosynthesis rate (e.g.Yan *et al.*, 2015).

1.4b. Strigolactones

Strigolactones (SLs), first characterized more than 45 years ago as stimulants for seed germination in root parasitic plants, such as Striga, Orobanche, and Phelipanche species, are a very small group of carotenoid-derived compounds (e.g. Xiaonan, Kaori and Koichi, 2010). Later on, it was reported that SLs had the ability to induce arbuscular mycorrhizal fungi (AMF) hyphal branching. This root-derived signal was shown to enhance symbiosis between plants and AMF increasing the plant's root surface area. (Akiyama, Matsuzaki and Hayashi, 2005). More recently SL was reported to play a very significant role in plant architecture by inhibiting the outgrowth of axillary buds thus suppressing shoot branching (e.g. Umehara *et al.*, 2008). SLs are now well accepted as a renowned class of phytohormones considered to be of increasing importance to the science of plants. They are also called semiochemicals. Semiochemicals are a group of biologically active molecules that are used to disseminate information between individual species (Zwanenburg, Pospíšil and Ćavar, 2016). The first isolated form of SL was named strigol. Strigol was isolated in 1966 from the root exudates of cotton (Gossypium hirsutum L.) (Cook *et al.*, 1966). In the year 1972, the structure of strigol was coined and only later on in 1985 approximately two decades later was the full details of this structure by means of X-ray diffraction analysis determined (Brooks, Bevinakatti and Powell, 1985). There are a group of important plant species such as parasitic weeds witchweed (Striga spp., Orobanchaceae/Scrophulariaceae) and broomrape (Orobanche spp., Orobanchaceae that have become completely reliant on these allelochemicals (Zwanenburg, Pospíšil and Ćavar, 2016).

Recently, a good number of new bio-properties of SLs have been uncovered, for example, treatment with an exogenously applied SL, practically in so many cases so far studied with the application of the synthetic GR24, resulted in the inhibition of the branching of shoots (Dun *et al.*, 2012), internode growth stimulation (de Saint Germain *et al.*, 2013), the speeding up of leaf senescence (e.g. Yamada *et al.*, 2014), the increase in root hair elongation, the enhancement of primary root growth (Kapulnik *et al.*, 2011), inhibition of the outgrowth of axillary buds (Minakuchi *et al.*, 2010), inhibition of the formation of adventitious and lateral roots (e.g. Rasmussen, Christine and Geelen, 2012), increasing stem thickness and inducing secondary growth (Agusti *et al.*, 2012), etc. It was found that auxin–SL interactions at multiple levels is critical for branching control (Stirnberg, Ward and Leyser, 2010). Details at the molecular level on how these inhibitory processes operate are still undefined.

Already mentioned above a real groundbreaking discovery in the functioning of SLs as phytohormones was the discovery that, they can act as the branching factors for arbuscular mycorrhizal fungi (AMF) (Akiyama, Matsuzaki and Hayashi, 2005). This association is considered the most prevalent symbiosis on earth. The AMF are Fungi and are members of the phylum Glomeromycota. Most terrestrial vascular flowering plants form symbiotic associations with these Fungi group. These associations are beneficial

for plants especially those plants growing under suboptimal environmental conditions (Redecker and Raab, 2006). During this symbiosis association, the hyphae of AMF spread into the soil from the plant's roots, thus providing an increased surface area for the plant. This allows the plant to be able to access a larger volume of the soil through its roots and thereby increase the number of nutrients and water that can be accessed and taken up by the plant (Rausch and Bucher, 2002). In return, the fungus receives fixed carbon in the form of glucose, hexoses or sucrose from the host plant (Solaiman and Saito, 1997). A second and very significant groundbreaking discovery in the functioning of SLs as plant phytohormones, was its role in the inhibition of bud outgrowth and shoot branching thereby controlling plant architecture especially plants growing under the influence of environmental stress. (e.g. Umehara *et al.*, 2008). The inhibitory processes are controlled by endogenous signals of which SLs are perchance most prominent.

1.4b.1. Structure

SLs are usually made up of three connected rings that are joined together. These rings include the ABC scaffold, connected by means of an enol ether unit with a butenolide ring, called the D-ring. At the moment two families of naturally occurring SLs are known, alectrol and orobanchol, and a third example, solanacol, is considered as well. Naturally occurring SLs are generally not produced on a large scale. The reason being that they have a too complex structure for a multi-gram scale synthesis. So far, the study of the effects of SLs on different biological processes has only been made possible by the use of model compounds. A prerequisite for designing and preparing these model compounds (analogs) is that, they have a much simpler structure than the natural occurring SLs and that their bioactivity is largely retained. Additionally, they must be synthetically readily accessible. It is important that when designing and preparing a rational design of SL analogs the bioactiphore be identified. The bioactiphore of a molecule is that part of the molecule that is primarily responsible for its bioactivity (Zwanenburg, Pospíšil and Ćavar, 2016). Additionally, the stereochemistry should be taken into consideration when designing these SL analogs as it has been well demonstrated to have an influence on the germinating activity of parasitic weeds. So far, the structure of SL has been systematically simplified and is made up of three main compounds. Firstly, GR24 code-named after its inventor Gerald Rosebery is a product from making the A-ring aromatic. Secondly, GR7 is a product from the removal of the A-ring and thirdly, GR5 is a product from the cutting away of the B-ring (Figure 1.3). All these GR compounds are appreciably active as germination stimulants for parasitic weeds (Zwanenburg, Pospíšil and Ćavar, 2016).

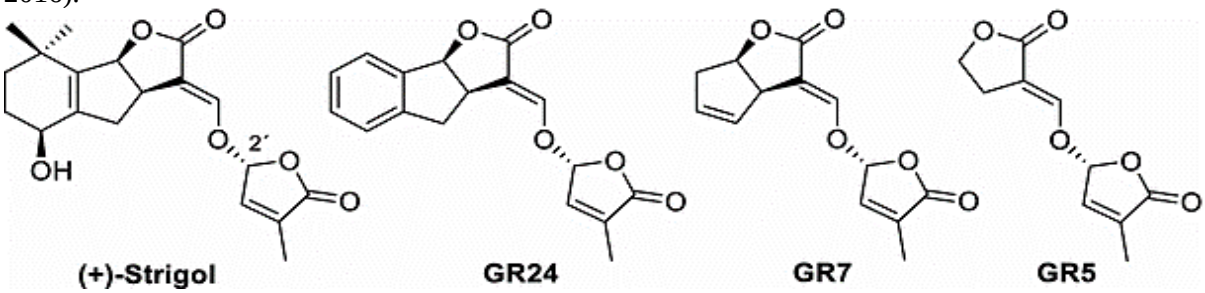


Figure 1.3: Simplified structures of (+)-Strigol. *GR24, GR7, and GR5 are all appreciably active as germinating agents (stimulants)*(Zwanenburg *et al.*, 2009).

1.4b.2. Synthesis and perception

Strigolactones are active at extremely low concentrations (e.g. Joel, 2000). Three Plastid localized proteins have been shown to be implicated in the first stages of Strigolactone biosynthesis and perception. This is a model that has been tested in higher plant species (Booker *et al.*, 2012). One of these plastid localized proteins is a carotenoid isomerase DWARF27 (D27). A protein that has been characterized in plant species like rice Arabidopsis and pea (Hao *et al.*, 2009; Alder *et al.*, 2012). D27 can convert all-trans b-carotene into 99-cis-b-carotene (Alder *et al.*, 2012). Carotenoid cleavage dioxygenase7 (CCD7) and CCD8, which are two bond specific enzymes, oxidatively tailor, cleave, and cyclize 99-cis-b-carotene (Booker *et al.*, 2005). After which a mobile intermediate, presumably the bioactive strigolactone precursor carlactone is formed. MORE AXILLARY GROWTH1 (MAX1), a class III cytochrome P450 monooxygenase has been suggested to be involved in the conversion of carlactone to strigolactone, a process that has not yet been fully characterized (e.g. Alder *et al.*, 2012; Booker *et al.*, 2005). SL has been suggested to be transported through the PLEIOTROPIC DRUG RESISTANCE1 (PDR1) protein upwards in the xylem and exuded into the rhizosphere and probably to neighboring cells. Kohlen *et al.*, (2011) presented evidence to this route of transportation of SL by presenting in Arabidopsis, proves of the presence of Strigolactone orobanchol, in the sap of its xylem.

Specific transporters have also been identified as other likely means for the transportation of Strigolactone. For example, Petunia hybrid ABC transporter PLEIOTROPIC DRUG RESISTANCE1, confined mainly in the region of the bud/leaf vasculature and subepidermal cells of the root is considered to be a cellular Strigolactone transporter. The strigolactone signal is suggested to be perceived by the F-box and Leu-rich repeats containing MORE AXILLARY GROWTH2 (MAX2) and also DWARF14 (D14) has been shown to play roles in the signal perception or transduction of this class of phytohormones (Stirnberg, van de Sande and Leyser, 2002; Stirnberg, Furner and Ottoline, 2007).

1.4b.3. Applications of SLs

SLs, their analogs and mimics have great potential for applications in agriculture. Modern-day agriculture requires a large amount of fertilization with phosphorus (P) from mineral sources to achieve high crop yields. The frequently fertilized P form is soluble Pi, which is readily accessible to the plant. The current use of Pi in agriculture is not sustainable mainly because global P sources are depleting. Additionally, the runoff of P into streams and rivers causes eutrophication. Importantly, plants can only make use of about 10% to 25% of the fertilized Pi due to the fact that it diffuses very slowly and adheres to soil particles. Due to the just mentioned disadvantages of applying P, finding different substitutions for the use of P to increase plant production is very important. For example, enabling plants to be more efficient at absorbing dissolve Pi from the soil can be beneficial for the environment and crop yield simultaneously. This can be achieved by increasing the root surface area of plants which can be realized through lateral roots, root hairs, cluster roots, and mycorrhizal hyphae. SL- focused strategies if directed towards particular crops might increase the ability of plants to absorb Pi from the soil by promoting the formation of mycorrhiza and root hair growth. This can, therefore, be beneficial for increasing the availability and uptake of Pi for food production at the same time increasing the sustainability of crop production (Guowei *et al.*, 2017).

Secondly, the use of SLs for the control of parasitic weeds is under active investigation. For example, the suicidal germination approach. This is an approach in which the seed of weed is tricked to germinate in the field in the absence of the host plant. Here, an analog of SL is applied to the field in the absence of the host plant. The seeds of the weed germinate due to the signal given off by the presence of SL, but because there is no actual host to give them nutrients, they die as a result. After the death of the parasitic weed, the host plant (an important crop) can then be planted on the field (Zwanenburg *et al.*, 2009).

Thirdly, the symbiotic role of AMF with parasitic plants. After this was first observed, much attention was given to the beneficial mutualistic and symbiotic associations of AM fungi and parasitic plants (Bonfante and Requena, 2011). AM fungi serve as soil fertilizer by facilitating the uptake of phosphates and nitrates. An in-depth understanding of this symbiotic relationship could provide new ways to control beneficial fungal symbionts as well as minimize the devastating effects of parasitic weeds in agriculture and natural ecosystems. Importantly, recent research on the enhancement of drought tolerance in WT plants has indicated that there is a great potential for the use of genetic engineering to increase the stress tolerance of crop plants by influencing the biosynthesis and/or signaling of SL (Garg and Singla, 2011).

1.5. Wheat

With the present production of ~ 700 Mt, wheat is the third principal crop worldwide and an important source of calories in human diets. It is the most significant cereal crop for the majority of the world's population (Marconi and Carcea, 2001), and the most important staple food of about two billion people (36% of the world population). 55% of the carbohydrates and 20% of the food calories consumed globally is offered by wheat. Wheat stands out exceeding the average production of other major grain crops like rice and maize thereby, considered the most significant cereal crop cultivated across a wide range of climatic conditions across the world (Baquar, 1989). Common wheat belongs to the Kingdom: Plantae, Order: Poales, Family: Poaceae. It is a grass that has an annual cycle: with simple culms, growing erect up to 1.2 m tall (Briggle, 1981). It provides for our bodies a very complex kind of carbohydrates and vitamins as well. These carbohydrates serve as the best fuel for our bodies because they are low in fat and high in vitamins. Additionally, they also provide a very special group of vitamins called the 4 key B vitamins. Medicinally, wheat has been used to cure a number of different liver conditions, psoriasis etc. (Jain and Argal, 2014). It is also claimed to improve digestion, reduce high blood pressure as it enhances the capillaries, support the growth of lactobacilli and can remove HMs from the body (Marwaha *et al.*, 2004). Furthermore, hematological toxicity, a condition that arises in breast cancer patients undergoing chemotherapy, is one that can be improved with the use of certain

components from wheat. Also, patients of the rear blood disease thalassemia major reduce the number and frequency of blood transfuse ions they need by consuming wheat products (Bar-Sela *et al.*, 2007). With all that said, wheat is generally accepted as a very important cereal, a key component of the human diet and health as well. Because of the complexity of studying its grains, the great number of potential health-promoting components present in there are unidentified and still under debate. In this study, we used wheat, which is well known to be a non-metallophyte to investigate the possible effects of the HMs Cu and Mn and the involvement of SL and JA on its growth improvement and HM stress tolerance.

In this study, we looked at a good number of plant parameters of wheat, for example, root length and weight and photosynthesis. But one which was of significant importance was the cellular tolerance test. This was a test that involved the evaluation of the cellular tolerance of the cells of the leaves of wheat plants by using plasmolysis.

1.6. Cellular tolerance

When commencing a study with the hope to fully understand the scope at which environmental pressures affect plant growth and development, it is always logical to start from the plant cell (Wei *et al.*, 2016). In plant physiology, the growth and development of plants are structured to allow the plant cell to enable the plant to respond to current environmental pressures while redirecting the structural context through which other stimuli still to come will be experienced (Dinneny, 2014). In this study, plasmolysis was used as a tool to measure the sensitivity and resilience of the cells of wheat leaves to HMs ($MnSO_4$ and $CuSO_4$ in solution).

In most mature higher plant cells, the living protoplasm environs a large aqueous central vacuole and forms a thin layer, between the cell wall and vacuole. The protoplasm layer includes two membranes: the plasmalemma (or cell membrane) and the tonoplast, which restrict the protoplasm from the cell wall and from the vacuole, separately. The vacuole and protoplasm layer (collectively called the protoplast) form a nearly ideal osmotic system for the reason that, the membranes possess a high degree of differential permeabilities. When a plant cell finds itself plunged into a hypertonic nonpermeating solute (e.g., sugars or mannitol), the vacuole and to a slight extend the protoplast loses water. This water loss from the cell continues until the water potential in the cell equals that on the outside. This causes a decrease in the turgor pressure brought about by cellular water loss. This results in a decrease of the protoplast volume occupied mainly by the vacuole and thus separation of the protoplast from the cell wall. This separation of the living protoplast from the cell wall resulting from water-withdrawing solutions (plasmolytica) is called plasmolysis (Lee-Stadelmann and Stadelmann, 1989).

❖ Questions pertaining to the cellular tolerance test.

In this study we expected the cellular tolerance test to answer the following questions about the cellular tolerance of the treatments considered.

- Do the cells of the respective HM treatments show more or less resilience to the respective HM in solution relative to the C treatment?
- Do the cells of the respective phytohormone treatments show more or less resilience to the respective HM in solution relative to the C treatment?
- Do the cells of the respective HM/ phytohormone treatments show more or less resilience to the respective HM in solution relative to the C treatment.

1.7. Objectives and Questions

This study was carried out in a two-phased experimental design in which, the possible involvement of two phytohormones MeJA (JA) and GR24 (SL) on the stress tolerance of wheat plants to $CuSO_4$ (Cu) and $MnSO_4$ (Mn) was investigated. The first objective was the same for both experiments because the same type and concentrations of HMs were used meanwhile, the second and third objectives were not the same for both experiments because two different types of phytohormones were applied, each for the respective experiments.

- **Objective one**

To investigate the effects of different concentrations of HM ($CuSO_4$ and $MnSO_4$) on wheat.

- **Objective two**
  a. To investigate the possible involvement of JA on the growth performance of wheat.
  b. To investigate the possible involvement of SL on the growth performance of wheat.
- **Objective three**
  a. To investigate the possible involvement of JA on the HM stress tolerance of wheat.
  b. To investigate the possible involvement of SL on the HM stress tolerance of wheat.

❖ Questions pertaining to treatment groups.

The following questions break down the main objectives of this study:

- What is the effect of the different concentrations of Cu on the different plant parameters measured?
- What is the effect of the different concentrations of Mn on the different plant parameters measured?
- Is there a possible involvement of JA on the different plant parameters of the Phytohormone treatments?
- Is there a possible involvement of JA on the different plant parameters of Cu/phytohormone treatments?
- Is there a possible involvement of JA on the different plant parameters of Mn/phytohormone treatments?
- Is there a possible involvement of SL on the different plant parameters of the phytohormone Treatments?
- Is there a possible involvement of SL on the different plant parameters of the Cu/phytohormone treatments?
- Is there a possible involvement of SL on the different plant parameters of the Mn/phytohormone treatments?

2. Materials and methods

Biological controlled wheat seeds from Nestelberger (AT-BIO-301) were seeded into 60 ml pots containing approximately 22 g of soil composed of a homogenized mixture of 25% clay, 14% silt, and 61% sand. This soil was kindly provided for the study by the gardeners of the University of Vienna. A total number of five seeds for the first experiment and eight seeds for the second experiment was sown into the soil which was hitherto treated with different concentrations of Cu (10^{-3} M, $5.10^{-}3$ M, and $10^{-}2$ M) and Mn (10^{-3} M, 10^{-2} M, and 5.10^{-2} M) respectively. Meanwhile, the five plants per pot stayed through till the end of the experiment of the first phase of this study, for the second phase, the plants were thinned down from eight to five plants per pot after a week of growth.

The plants of the respective experiments were subjected to heavy metal stress for a time period of approximately 5 weeks (36 days) for the first experiment and approximately 4 weeks (30 days) for the second experiment of this study. The plants were grown in the greenhouse at regularized temperatures variably ranging from 19.8°C to 34.1°C within the growing period. A preview of the experimental outlook of this study is provided in figure 2.1 below. Within the growth period of the respective experiments, the soil was weekly spiked by Cu and Mn. The aim of this was to ensure the constant replacement of HM leaking out of the pots in the progress of the respective experiments.

Picture a: week 1

Picture b: week 2

Picture c: week 3

Figure 2.1: Experimental outlook*. Pictures a, b and c provide an idea of the outlook of the experimental design of this study in progress of the first three weeks.*

2.1. Treatment with phytohormones

To investigate the possible involvement of the phytohormones on the HM induced stress, the plant's leaves were sprayed with phytohormones, JA for the first experiment, and SL for the second experiment, at concentrations of 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M respectively. In the first phase of this study, the stock solution and method of application of JA were replicated from Awang *et al.*, (2015) and the idea of what concentrations to be used was simulated from Van and Oomen, (2008); Yoon *et al.*, (2009); Yang *et al.*, (2012), in this experiment, the leaves of wheat plants were sprayed to wetness with JA from the second week, once weekly, and a total of four times by the end of the experiment. In the second phase of this study, the stock solution was prepared as simulated in Soto *et al.*, (2010) and the method of application of SL was simulated from Ha *et al.*, (2014). The idea of what concentrations to use was replicated from Gomez-Roldan *et al.*, (2008); Minakuchi *et al.*, (2010); Rasmussen *et al.*, (2013). In this experiment, the leaves of wheat plants were sprayed with 5ml of SL, a total of six times within the first three weeks of the experiment. In the progress of the respective experiment's plants were watered twice or thrice weekly with 20 or 30ml of water depending on the need for water by the plants. The plants were also fertilized once weekly with either 20 or 30 ml of WUXAL R super (comprising 8% N, 8% P_2O_5 and 6% k_2O), kindly provided by the gardeners of the University of Vienna.

2.2. Testing plant physiology

During the third week of the respective experiments, cellular tolerance of the plant's leaves was measured by the method of plasmolysis. In the last week of the respective experiments, prior to harvesting the plants, the chlorophyll fluorescence of the leaves was measured using a HANDY PEA meter, by following the instructions from the instrument's manual "the Hansatech instruments HANDY

PEA: Field reference guide". The value of the maximal fluorescence and the variable fluorescence were expressed as the FV/FM ratio of the leaves which served as a measure for the photosynthetic capacity of the plant leaves.

2.3. Testing plant morphology

At the end of the respective experiments, plants were taken out of the pots and the roots washed cautiously with distilled water. Subsequently, growth parameters such as root length, shoot length were measured in centimeter (cm), and the number of leaves counted. The plant tissue was oven-dried at 45^{o}C for two weeks after which shoots, and roots were separated and weighed in grams (g). For the statistical analysis and multiple comparisons of treatment groups, mean values of treatments were used to run the ANOVA test (Tukey unequal N HSD, $p < 0.05$) on the software Statistical Package for the Social Sciences (SPSS). Bar charts were used to report data and these bar charts show the calculated standard errors above which are letters indicating significant differences between treatment groups.

2.4. Testing cellular tolerance

Plasmolysis is a tool frequently used to test cell viability. Viable living cells can be plasmolyzed, and deplasmolyzed. The deplasmolysis is what actually confirms that the cell is 100% viable. Additionally, plasmolysis is an exclusive technique in experimental plant cell physiology to study the physicochemical properties of distinct plant cells and their variations in the living state (Lee-Stadelmann and Stadelmann, 1989). In this study, we used plasmolysis as a tool to measure the sensitivity and resilience of the leaf cells of wheat plants. Sections were abscised from the leaves of selected treatments and put into tiny cups containing graded concentrations of $CuSO_4$ and $MnSO_4$ in solution for a time period of 48hrs. The idea was to induce HM stress directly on the cells and then by using plasmolysis the cell's tolerance could be determined.

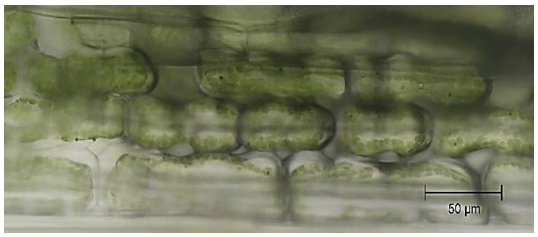
The treatments considered for the cellular tolerance test in this study were:

- The highest and lowest concentrations of the respective HM treatments.
- The highest and lowest concentrations of the respective phytohormone treatments.
- The highest and lowest concentrations of the respective HM/phytohormone treatments.
- Control treatments.

- **Technique of plasmolysis**

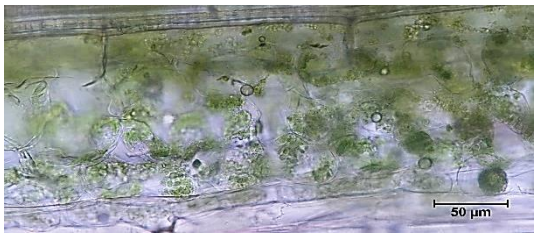
Wheat leaves of the various treatments considered for the cellular tolerance test were dissected into slices which were thick enough such that the epidermal cells remained alive. These tiny sections were imbibed in solutions of $MnSO_4$ and $CuSO_4$ in little white plastic cups, of concentrations 10^{-1} M to 10^{-6} M and tap water serving as a control respectively. These little cups were kept in the dark for 48hrs thereafter we checked the conditions of the cytoplasm in the light microscope (Olympus BX41). This was because it is often difficult to recognize if the cell is living or damaged. The withdrawal of the protoplast from the cell wall by hypertonic medium (80% of mannitol) is only possible if the cells are alive and the plasma membrane viable.

We categorized a cell to be plasmolyzed when we could observe under the light microscope that the protoplast completely shrinks away from the cell wall to the center of the cell (Figure 2.2a and b). On the other hand, a cell in this study was categorized to be unplasmolyzed based on a number of observable characteristics, for example, when it was observed under the light microscope that the protoplast did not shrink away from the cell wall or the chloroplast appeared unviable or the cell wall had burst open releasing the cellular content, in such cases, it was concluded that the cell was not viable and thus unplasmolyzed (Figure 2.2c and d). In this study, a section of the respective treatments was considered to be cellular tolerant when at least 50-90% of the cells were plasmolyzed (+), partially tolerant (+-) when not less than 10 to 40% of cells were plasmolyzed and unplasmolyzed (-), when < 5 % of cells were plasmolyzed.



Picture a

Picture b



Picture c

Picture d

Figure 2.2: Viable and dead cells as seen under the light microscope. Picture a *(treatment of JA10^{-5}M in CuSO4 solution of -10^{-5}M);* ***Picture b*** *(treatment of Cu 10^{-2}/JA 10^{-9} in solution CuSO$_4$ of 10^{-5}M);* ***Picture c*** *(treatment of JA 10^{-5} in CuSO$_4$ solution of 10^{-1}M);* ***Picture d*** *(treatment of Cu 10^{-2}/JA 10^{-9} in solution CuSO$_4$ 10^{-1}M).*

Treatments

This study was designed into two experiments consisting of treatment groups to enable easy evaluation of the results and answering of key questions. There was a total of 42 treatments per experiment and 3 replications per treatment.

- **Treatment groups**

- **The control treatment (C)**

These are the group of plants that were neither treated with HM (Cu or Mn) nor phytohormones (JA and SL) throughout the respective experiments.

- **Phytohormone treatment (JA and SL)**

These are plants that were grown in optimal environmental conditions in which the soil was not treated with HM (Cu or Mn) but whose leaves were sprayed with phytohormones (JA or SL) throughout the respective experiments.

- **HM treatment (Cu and Mn)**

These are plants that were grown in sub-optimal environmental conditions in which the soil was treated with HM (Cu or Mn) but whose leaves were not sprayed with phytohormones (JA or SL) throughout the respective experiments.

- **HM/Phytohormone treatments (Cu/JA, Cu/SL, Mn/JA, Mn/SL)**

These are plants that were grown in soil treated with HM (Cu or Mn) and whose leaves were sprayed with phytohormones (JA or SL) throughout the respective experiments.

- **Parameters measured**

- Root and shoot length in centimeters (cm)
- Root and shoot weight (dry weight) in grams (g)
- Chlorophyll fluorescence FV/FM ratio
- Number of leaves
- Cellular tolerance by plasmolysis

3. Results

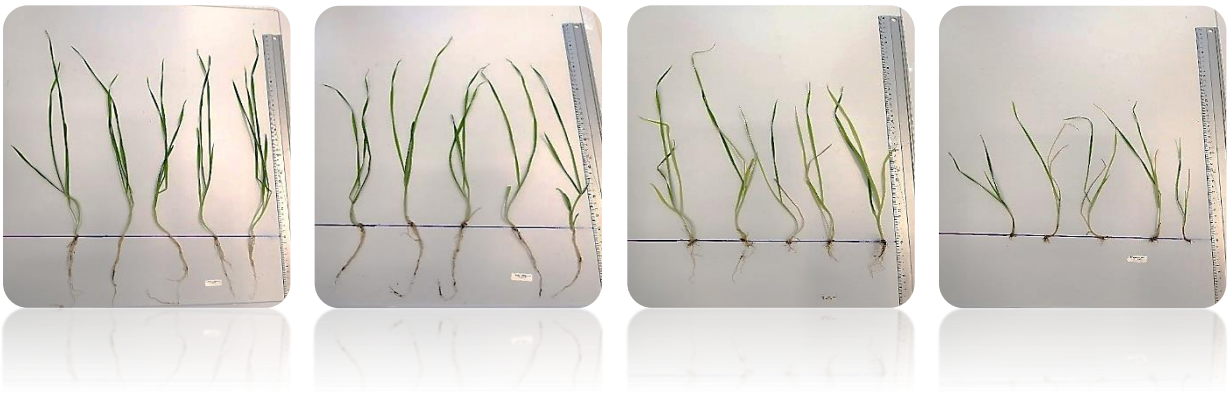
Wheat plants were subjected to heavy metal stress in a two-phase experiment of approximately five and four weeks respectively. Wheat seeds were seeded in 60 ml pots containing approximately 22 g of soil treated with Cu 10^{-3}, 5.10^{-3}, and 10^{-2} M and Mn 10^{-3}, 10^{-2}, and 5.10^{-2} M respectively. HM in solution was applied to the soil once weekly to ensure for the plant's roots, the continuous availability and replacement of heavy metals leaking out of the pots. To fortify the plants and enable them to cope with the abiotic stress induced by the HM in the soil, the plant's leaves were sprayed with different concentrations of phytohormones, JA for the first phase, and SL for the second phase of this study in concentrations of 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M and, 10^{-9} M respectively.

3.1. Effects of the HMs

3.1.1a. Effects of different concentrations of Cu on wheat

In this experiment typical symptoms of Cu, toxicity was showed by plants treated with Cu 5.10^{-3} and 10^{-2} M respectively. In these plants, abiotic stress was first seen on the leaves as depigmentation of chlorophyll at the tips of young leaves and this depigmentation extended downward along the leaf margins. The leaves were also twisted or malformed and showed chlorosis (i.e. loss of chlorophyll) or even necrosis (i.e. dying spots on the leaves). The roots of the Cu treated plants at these concentrations were short and looked stunted (greatly lignified) with very little or no root hairs and lateral root formation. The roots did not grow into the soil but remained suspended in the upper surface of the soil. The leaves of the plants treated with Cu 5.10^{-3} M and Cu 10^{-2} M were chlorotic and reduced in number and total surface area.

On the other hand, Cu 10^{-3} M treatment did not adversely decrease the growth parameters of the plants as shown in the Cu 10^{-2} M treated plants. Cu 10^{-3} M did not induce significant abiotic stress on the plants in comparison with the C. The Cu 10^{-3} M plants showed tolerance at this concentration with very little negative effects on the plant's parts. In general, parameters such as root and shoot weight, root and shoot length, decreased with the Cu 10^{-3} M treated plants but not significantly in comparison with the C treatment (Figure 3.1).



a.) C b.) Cu 10^{-3} M c.) Cu 5.10^{-3} M d.) Cu 10^{-2} M

***Figure 3.1**: **C and Cu treated plants in comparison.** The observable physical changes in roots and shoots when different concentrations of HM treated plants are compared to the C plants. **(**a) C is the control; these are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants were treated with increasing concentrations of Cu at concentrations of; (b) Cu 10^{-3} M, (c) Cu 5.10^{-3} M and (d) Cu 10^{-2} M respectively.*

3.1.1b. Different concentrations of Cu induce different levels of abiotic stress on wheat plants, affecting the root and shoot length

In this study, Cu treated plants were generally decreased in shoot length and root length in comparison with C plants and this relationship was linearly coordinated with the concentration of Cu applied to the soil. The roots and shoots of Cu 10^{-3} M treated plants were insignificantly affected by the presence of Cu in the soil in comparison to the C. On the other hand, Cu 5.10^{-3} M and Cu 10^{-2} M treated plants showed significant decreases in the shoot and root length in comparison to the C (Figure 3.2**).**

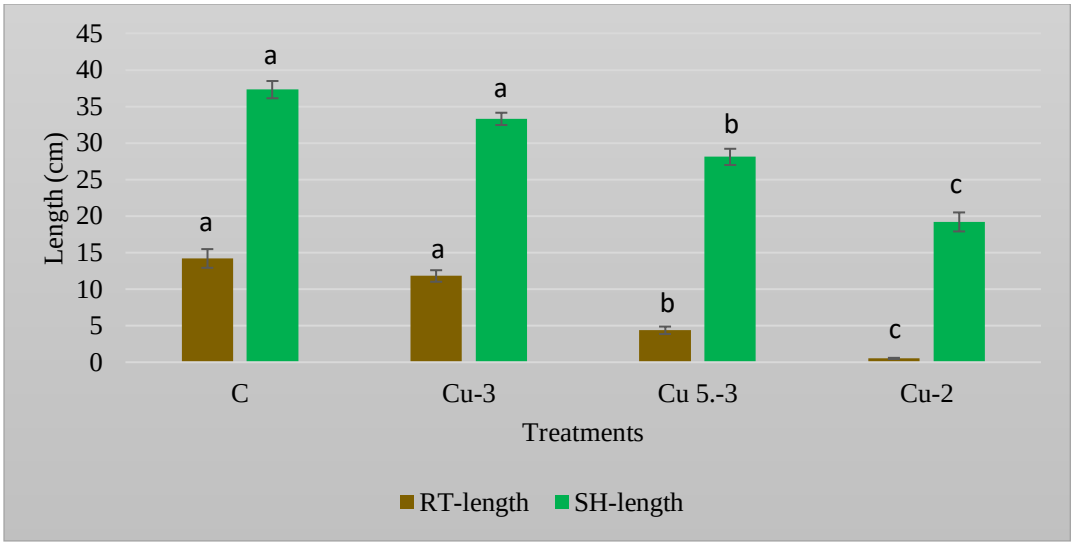


***Figure 3.2**: **Root and shoot lengths of C and Cu treated plants in comparison.** Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants were grown in soil treated with increasing concentrations of Cu at molar concentrations of Cu 10^{-3} (Cu-3), Cu5.10^{-3}(Cu 5.-3) and Cu 10^{-2}(Cu-2) respectively**.** Brown bars show the root length and green bars show the shoot length. Letters over the bars indicate significant differences between the mean value of root and shoot length between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences while different letters indicate significant differences between treatments* respectively.

3.1.1c. Plant dry weight indicates different levels of stress-induced by different concentrations of Cu treatments

Plants treated with Cu showed significant decreases in the root and shoot biomass especially at the highest concentration of Cu 5.10^{-3} M and Cu 10^{-2} M as compared to the C treatment respectively. The decrease in shoot and root biomass was directly proportional to the concentration of Cu applied to the soils. On the other hand, in comparison to the C treatments, the Cu 10^{-3} M treatments showed an insignificant reduction in the shoot weight, whereas the root weight was significantly decreased (Figure 3.3).

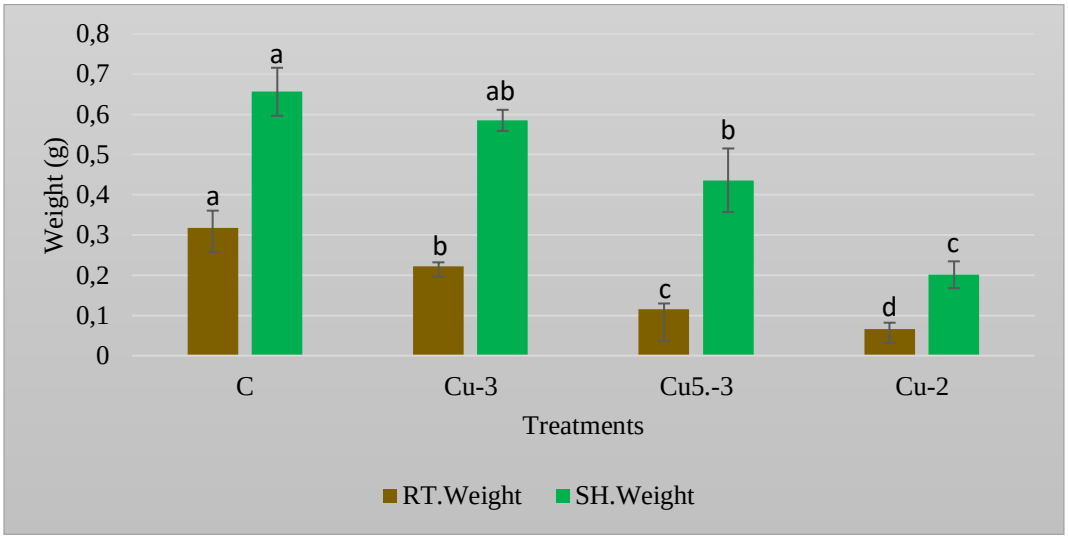


***Figure 3.3**: **Root and shoot dry weights of C and Cu treated plants in comparison.** Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants are grown in soil treated with increasing concentrations of Cu at molar concentrations of Cu 10^{-3} (Cu-3), Cu 5.10^{-3}(Cu5.-3) and Cu 10^{-2} (Cu-2). Brown bars show the root biomass and green bars show the shoot biomass. Letters over the bars indicate significant differences between the mean value of root and shoot biomass between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). similar letters over the bars indicate no significant differences, while different letters indicate significant differences between the treatments.*

3.1.1d. Different concentrations of Cu induce different levels of stress on the photosynthetic ability of wheat leaves

In this experiment, the FV/FM ratio either increased insignificantly or decreased significantly depending on the Cu concentration applied to the soil in comparison with the C treatment. Plants in soils treated with Cu 10^{-3} M showed an insignificant increase in the FV/FM ratio when compared to C plants, whereas plants treated with Cu 5.10^{-3} M and Cu 10^{-2} M showed a significant decrease in the FV/FM when compared to the controls (Figure 3.4).

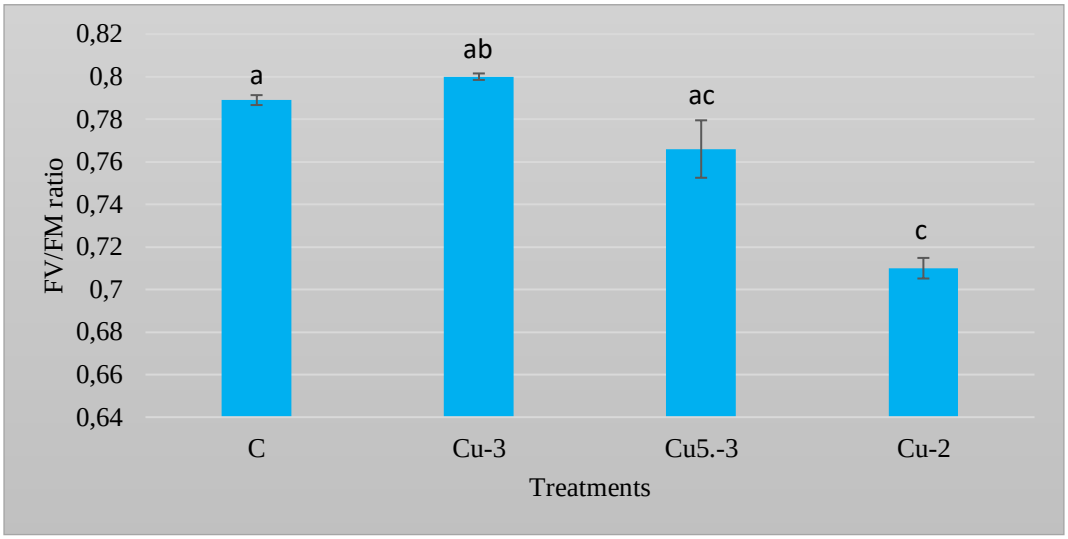


***Figure 3.4*: FV/FM ratio of C and Cu treated plants in comparison.** *Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants are grown in soil treated with increasing concentrations of Cu at molar concentrations of Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2}(Cu-2). Blue bars indicate the mean value of the fluorescence measurement of the leaves (The FV/FM ratio). Letters over the bars indicate significant differences between the mean value of the FV/FM ratios between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences while different letters indicate significant differences between the treatments.*

3.1.1e. Different concentrations of Cu affect variably, the ability of wheat plants to form leaves

In comparison with the C plants, Cu insignificantly increased the total number of leaves formed by the plants treated with Cu 10^{-3} and Cu 5.10^{-3} M respectively. The Cu 10^{-2} M concentration applied to the soil showed a significant decrease in the number of leaves formed by the plants in comparison to the controls (Figure 3.5).

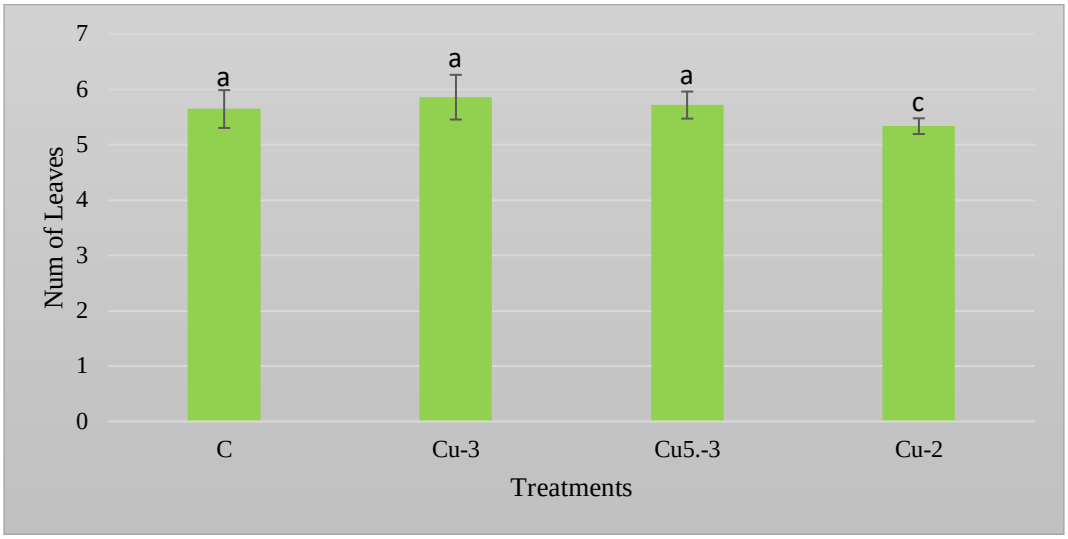
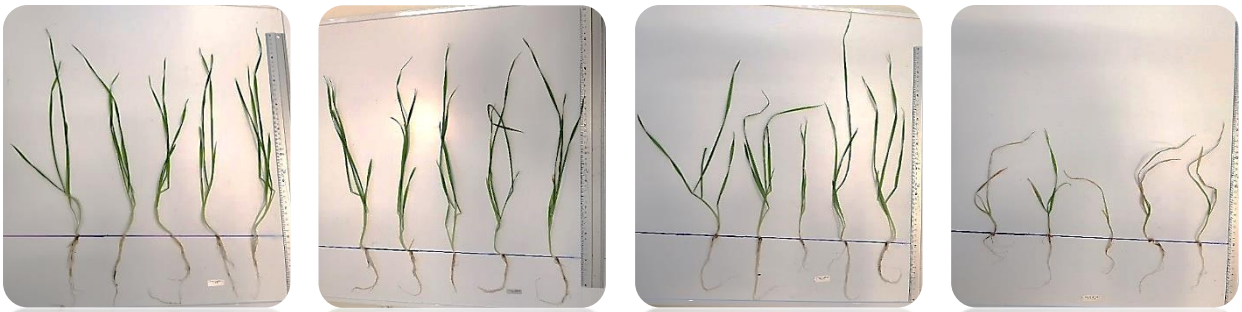


***Figure 3.5**: **The Number of leaves of C and Cu treated plants in comparison.** Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants are grown in soil treated with increasing Molar concentrations of Cu at molar concentrations of Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)**.** Green bars indicate the mean value of the number of leaves counted per plant (Num of leaves). Letters over the bars indicate significant differences between the mean value of the Num of leaves between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences while different letters indicate significant differences between the treatments.*

3.1.2a. Effects of different concentrations of Mn on wheat

In this experiment, the concentrations of Mn selected variably acted as essential macronutrients Mn 10^{-3} M, to minimally toxic 10^{-2} M and significantly toxic 5.10^{-2} M. Improvement in growth parameters such as root length, shoot length, and the biomass of roots and shoots were detected for the group of plants treated with Mn 10^{-3} M in comparison to the C. In addition to a reduction in growth rate, symptoms of Mn toxicity such as chlorosis in leaves and necrotic leaf spots were very common observations in plants treated with Mn 10^{-2} M and mostly in plants treated with Mn 5.10^{-2} M. Generally, growth parameters such as root and shoot length, root and shoot dry weight, the number of leaves and FV/FM ratio decreased with an increase in the concentration of Mn treatments in comparison with C (Figure 3.6).



a.) C b.) Mn 10^{-3} M c.) Mn 10^{-2} M d.) Mn 5.10^{-2} M

***Figure 3.6**: **C and Mn treated plants in comparison.** The observable physical changes in roots and shoots when different concentrations of Mn treated plants are compared to the C plants. **(**a) C is the control; these are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants were treated with increasing concentrations of Cu at concentrations of; (b) Mn 10^{-3}M, (c) Mn 10^{-2} M and (d) Mn 5.10^{-2}M respectively.*

3.1.2b. Different concentrations of Mn induce different levels of abiotic stress on wheat plants thus affecting the root and shoot length

In this experiment, plants treated with Mn 10^{-3} M showed insignificant increases in the root length and shoot length in comparison with the C and, Mn 10^{-2} M showed insignificant decreases in the shoot length and significant decreases in the root length in comparison with the C. On the other hand, Mn 5.10^{-2} M was significantly toxic to the plants. Treatments at this concentration showed significant decreases in root and shoot length in comparison with the C (Figure 3.7**).**

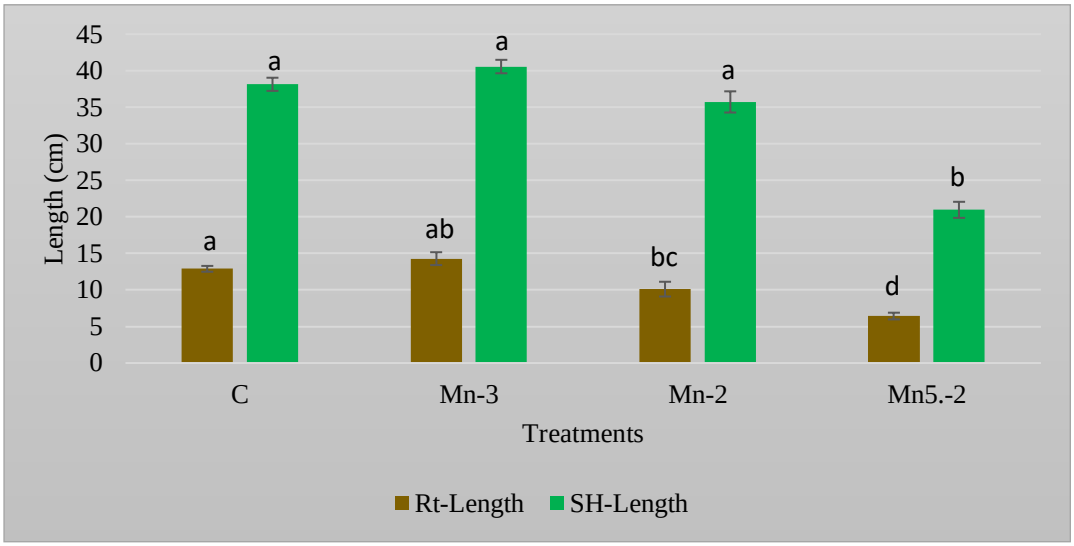


***Figure 3.7**: **Root and shoot lengths of C and Mn treated plants in comparison.** Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants are grown in soil treated with increasing concentrations of Mn at molar concentrations of Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2). Brown bars show the root length and green bars show the shoot length. Letters over the bars indicate significant differences between the mean value of root and shoot length between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). similar letters over the bars indicate no significant differences while different letters indicate significant differences between treatments.*

3.1.2c. Plant dry weight indicates different levels of stress-induced by different concentrations of Mn treatments

In this experiment, the roots of Mn treated plants reduced when compared to the C plants. The shoots, on the other hand, varied from being insignificantly increased at the lowest concentrations, to being significantly decreased at the highest concentrations of Mn treatments respectively. For the Mn 10^{-3} M, the roots were insignificantly decreased in comparison to the C. On the other hand, the roots of Mn 10^{-2} M and Mn 5.10^{-2} M were significantly decreased in comparison to the C. For the shoot dry weight, the treatments with Mn 10^{-3} M and Mn 10^{-2} M were insignificantly increased in comparison to the C. Whereas the treatment with Mn 5.10^{-2} M significantly decreased the shoot biomass of the plants in comparison to the C (Figure 3.8).

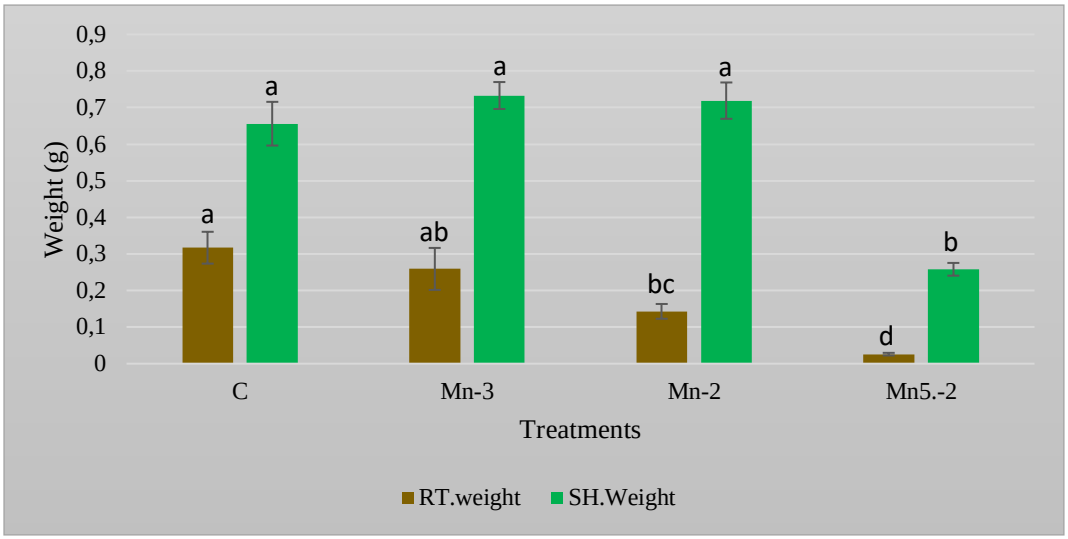


***Figure 3.8**: **Root and shoot dry weights of C and Mn treated plants in comparison.** Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants are grown in soil treated with increasing concentrations of Mn at molar concentrations of Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2)**.** Brown bars show the root biomass and green bars show the shoot biomass. Letters over the bars indicate significant differences between the mean value of root and shoot biomasses between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). similar letters over the bars indicate no significant differences while different letters indicate significant differences between treatments.*

3.1.2d. Different concentrations of Mn induce different levels of stress on the photosynthetic ability of wheat leaves

Mn in this experiment significantly decreased the photosynthetic rate at higher concentrations of Mn applied to the soil. At lower concentrations of Mn in the soil there was an insignificant increase in photosynthetic rate. Mn 10^{-3} M showed an insignificant increase in the FV/FM ratio, on the other hand, Mn 10^{-2} M showed an insignificant decrease in the FV/FM ratio when compared to the C treatments respectively. Mn 5.10^{-2} M showed a significant decrease in the FV/FM ratio when compared to the C treatment (Figure 3.9).

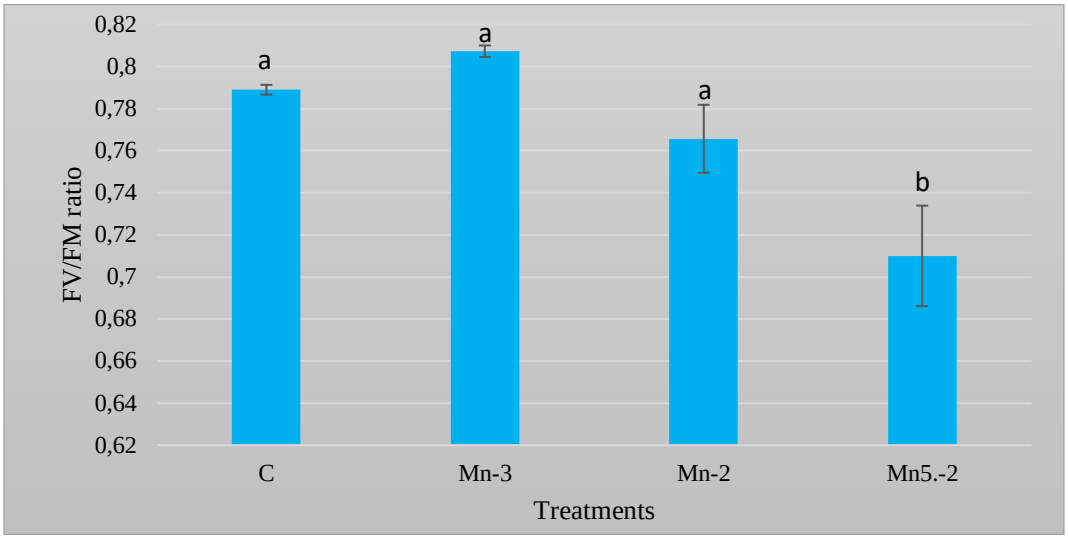


***Figure 3.9**: **FV/FM ratio of C AND Mn treated plants in comparison.** Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants were grown in soil treated with increasing concentrations of Mn at Molar concentrations of Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2). Blue bars indicate the mean value of the fluorescence measurement of the leaves (the FV/FM ratio). Letters over the bars indicate significant differences between the mean value of the FV/FM ratios between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences while different letters indicate significant differences between the treatments.*

3.1.2e. Different concentrations of Mn affect variably, the ability of wheat plants to form leaves

In this study, Mn 10^{-3} and Mn 10^{-2} M treatments showed insignificant increases in the total number of leaves formed by the plants whereas the plants exposed to soils treated with Mn 5.10^{-2} M, showed significant decreases in the number of leaves formed when compared to the C plants respectively (Figure 3.10).

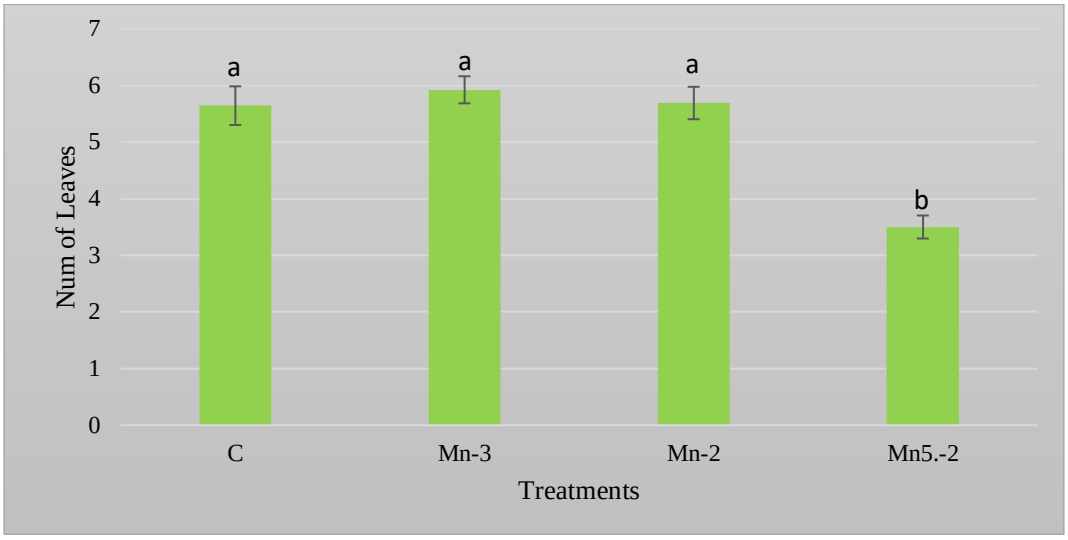


Figure 3.10: The number of leaves of C and Mn treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM throughout the experiment. Wheat plants were grown in soil treated with increasing concentrations of Mn at molar concentrations of Mn 10^{-3} (Mn-3), Mn $10^{(-2)}$ (Mn (-2)) and Mn 5.10^{-2} (Mn5.-2. Green bars indicate the mean value of the number of leaves counted per plant (the Num of leaves). Letters over the bars indicate significant differences between the mean value of the Num of leaves between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences while different letters indicate significant differences between the treatments*

3.2. Effects of phytohormones

In this study, it was with absolute certainty established that Cu and Mn induced abiotic stress on wheat plants at different degrees. This was dependent on the concentration of the respective HMs applied to the soil; therefore, we applied the phytohormones. Furthermore, to fully understand the role played by the phytohormones on the wheat plants, we investigated first the influence of the application of phytohormones on plants grown in optimal environmental conditions. To this end, phytohormones were applied to find out if:

a. The phytohormones had any possible influence on the parameters of the phytohormone treatments; plants growing in optimal environmental conditions. To do this the respective parameters of the **phytohormone treatments** were compared to the parameters of the **C treatment**.
b. The phytohormones had any possible influence on the parameters of the heavy metal treatments; plants grown in sub-optimal environmental conditions. To do this the respective parameters of the **HM/phytohormone** treatments were compared to the **HM treatments**.

By running multiple comparison test with ANOVA, only significant differences between treatments were considered in this study to indicate a potential influence of the phytohormones.

3.2.1. The involvement of phytohormones on wheat plants grown in optimal environmental conditions

3.2.1a. The involvement of JA on root and shoot length of phytohormone treatments

JA, concentrations of 10^{-5} M and 10^{-6} M significantly decreased the root length of the phytohormone treatments in comparison with the C plants. On the other hand, the concentrations of 10^{-6} M, 10^{-7} M and 10^{-8} M of JA significantly increased the shoot length of the wheat plants not induced by HM stress, in comparison with the C treatment respectively (Figure 3.11).

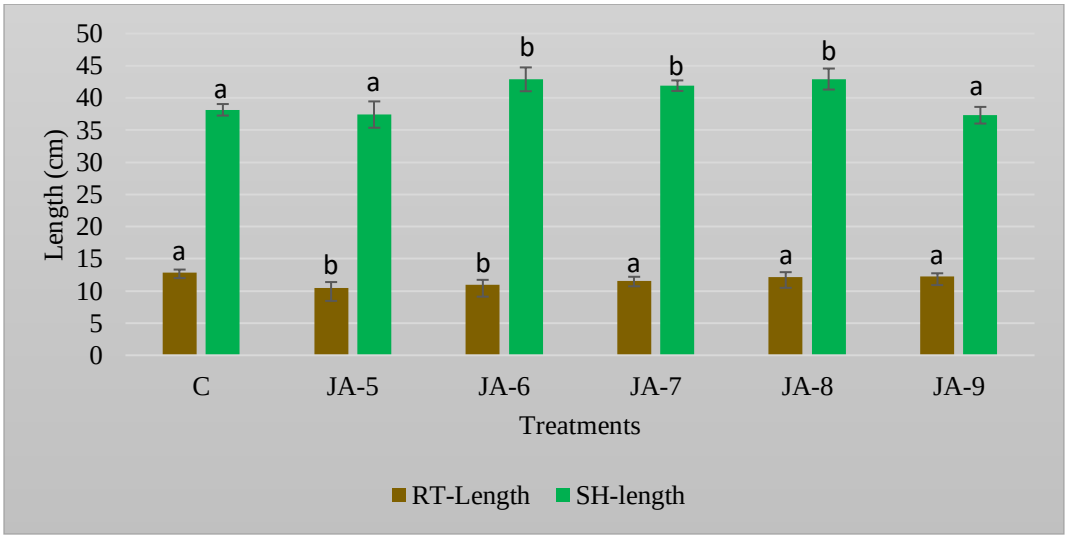


Figure 3.11: Root and shoot length of C and JA treatments in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no JA application throughout the experiment. Wheat plants sprayed with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, are labeled on the charts as JA-5, JA-6, JA-7, JA-8, and JA-9 respectively. Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Letters over the bars indicate significant differences between the mean value of the shoot and root length of all JA treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between JA treated plants in comparison with the C treatment, while different letters indicate significant differences between JA treated plants in comparison with, the C treatment.*

3.2.1b. The involvement of JA on root and shoot weight of phytohormone treatments

JA, 10^{-5} M significantly decreased the root weight of the phytohormone treatments in comparison with the C., On the other hand, no significant influence of all the applied concentrations of JA was shown by the shoot weight. (Figure 3.12)

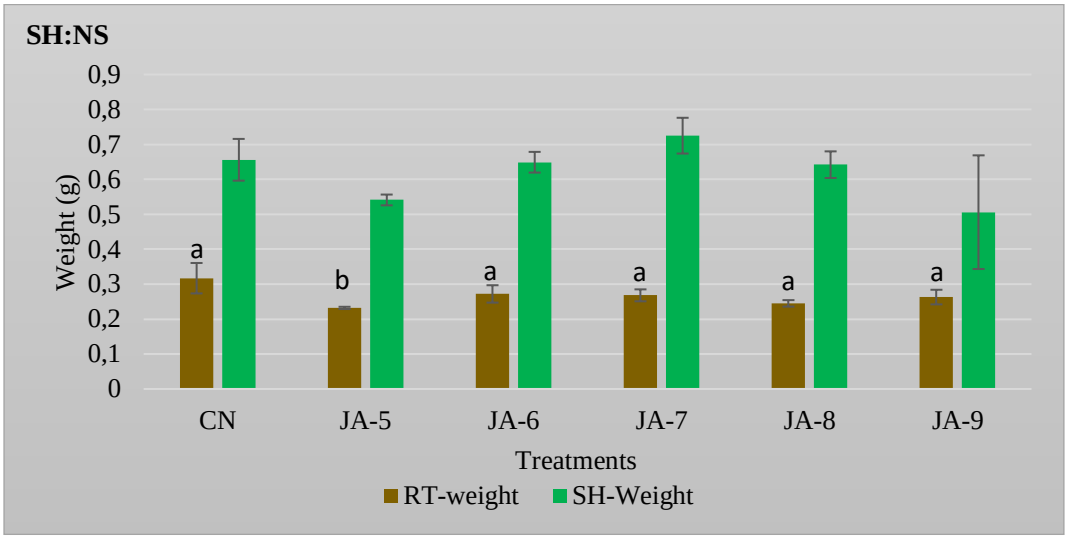


Figure 3.12: Root and shoot weight of C and JA treatments in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no JA application throughout the experiment. Wheat plants sprayed with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations are labeled on the graph as JA-5, JA-6, JA-7, JA-8, and JA-9 respectively. Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Letters over the bars indicate significant differences between the mean value of the shoot and root weight of all JA treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between JA treatments in comparison with the C treatment, while different letters indicate significant differences between JA treatments in comparison with, the C treatment.*

3.2.1c. The involvement of JA on FV/FM ratio of phytohormone treatments

All concentrations of JA applied showed no significant involvement in the FV/FM ratio of the leaves of the phytohormone treatments (Figure 3.13).

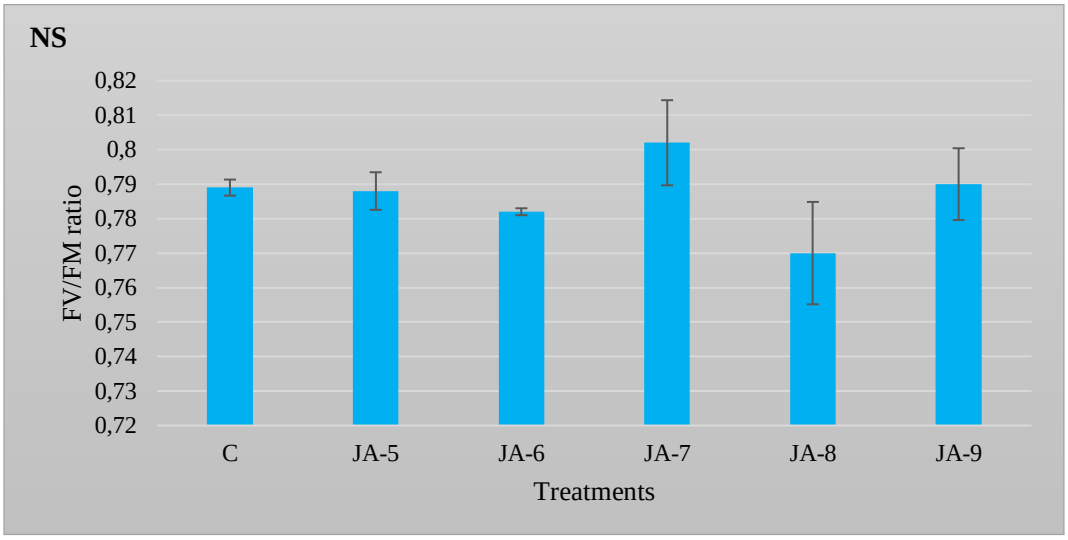


Figure 3.13: FV/FM ratio of C and JA treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no JA application throughout the experiment. Wheat plants sprayed with molar concentrations of JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations are labeled on the graph as JA-5, JA-6, JA-7, JA-8, and JA-9 respectively. Blue bars indicate the mean value of the FV/FM ratio. Letters over the bars indicate significant differences between the mean value of the FV/FM ratio of all JA treatments in comparison with the C treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between JA treatments in comparison with the C treatment, while different letters indicate significant differences between JA treatments in comparison with, the C treatment.*

3.2.1d. The involvement of JA on the number of leaves of phytohormone treatments

There is no observation of a significant influence of JA on the number of leaves formed by the JA treatments (Figure 3.14)

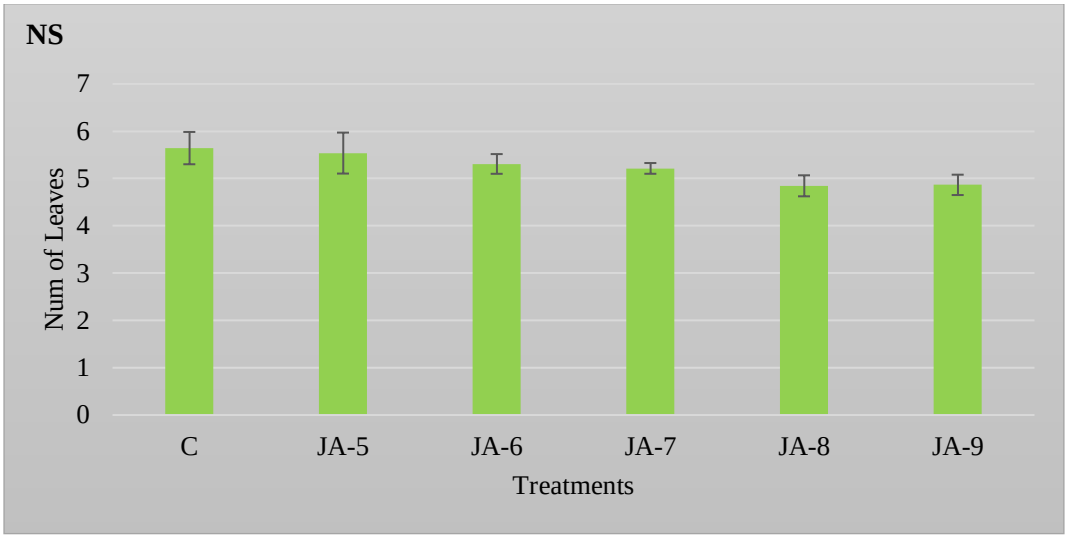


Figure 3.14: The number of leaves of C and JA treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no JA application on the leaves throughout the experiment. Wheat plants sprayed with JA 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} molar concentrations are labeled on the graph as JA-5, JA-6, JA-7, JA-8, and JA-9 respectively. Green bars indicate the mean value of the number of leaves. Letters over the bars indicate significant differences between the mean value of the number of leaves of all JA treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate insignificant differences between JA treated plants in comparison with the C treatment, while different letters indicate significant differences between JA treated plants in comparison with, C treatment.*

3.2.1e. The involvement of SL on root and shoot length of phytohormone treatments

All concentrations of SL applied to the phytohormone treatment plants showed no significant involvement in the root and shoot length of the plants (Figure 3.15).

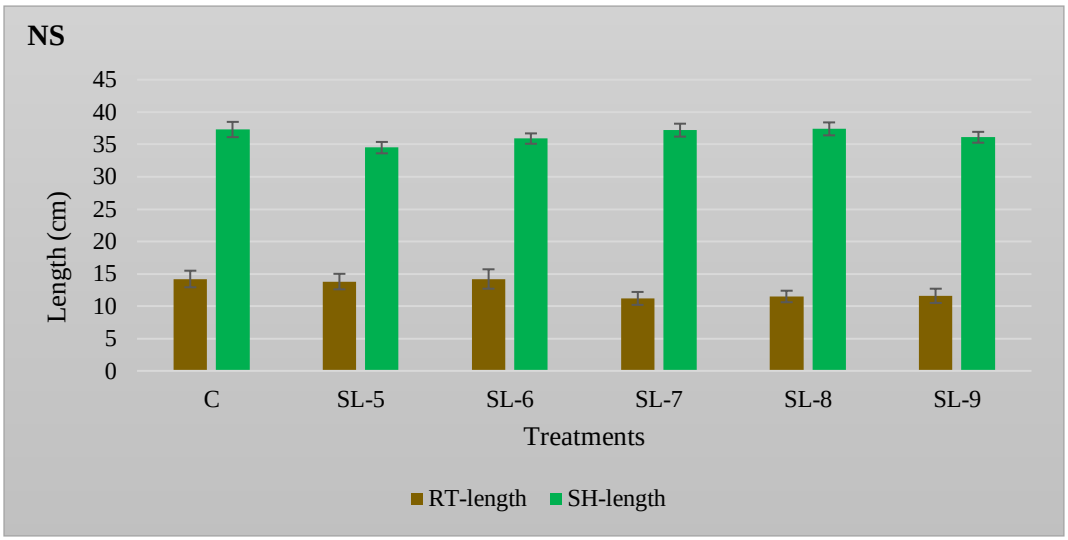


Figure 3.15: Root and shoot length of C and SL treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no SL application throughout the experiment. Wheat plants sprayed with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, are labeled on the graph as SL-5, SL -6, SL -7, SL -8, and SL -9 respectively. Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Letters over the bars indicate significant differences between the mean value of the shoot and root length of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between SL treated plants in comparison with the C treatment, while different letters indicate significant differences between SL treated plants in comparison with, the C treatment.*

3.2.1f. The involvement of SL on root and shoot weight of phytohormone treatments

SL 10^{-7} M significantly decreased the root weight of the phytohormone treatment plants. On the other hand, SL 10^{-8} M significantly increased the shoot weight of the phytohormone treatment plants (Figure 3.16).

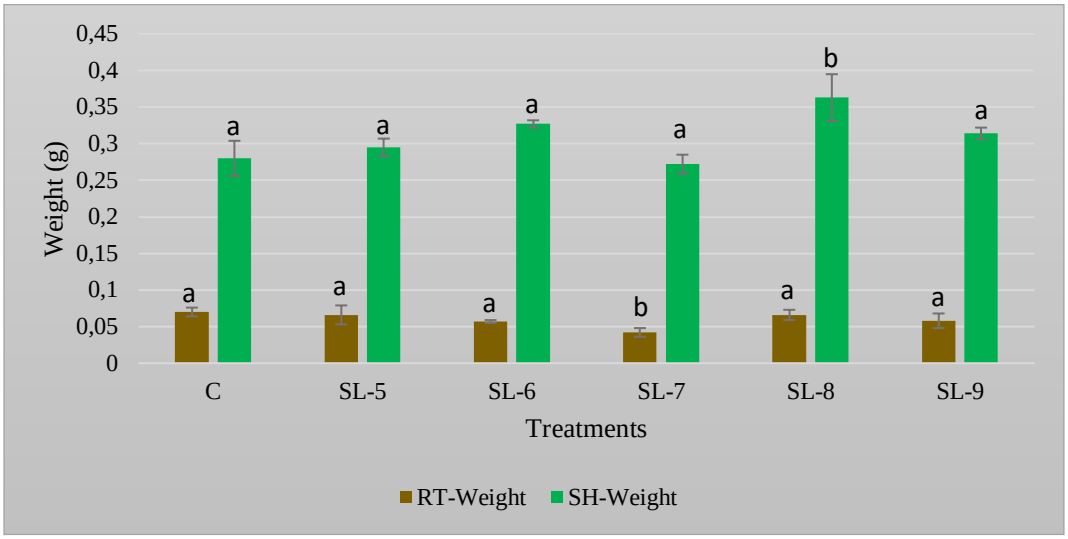


Figure 3.16: Root and shoot weight of C and SL treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no SL application throughout the experiment. Wheat plants sprayed with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations are labeled on the graph as SL-5, SL -6, SL -7, SL -8, and SL -9 respectively. Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Letters over the bars indicate significant differences between the mean value of the shoot and root weight of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between SL treated plants in comparison with C, while different letters indicate significant differences between SL treated plants in comparison with the C treatment.*

3.2.1g. The involvement of SL on the FV/FM ratio of the phytohormone treatments

SL showed No significant influence on the FV/FM ratio of the phytohormone treatments (Figure 3.17).

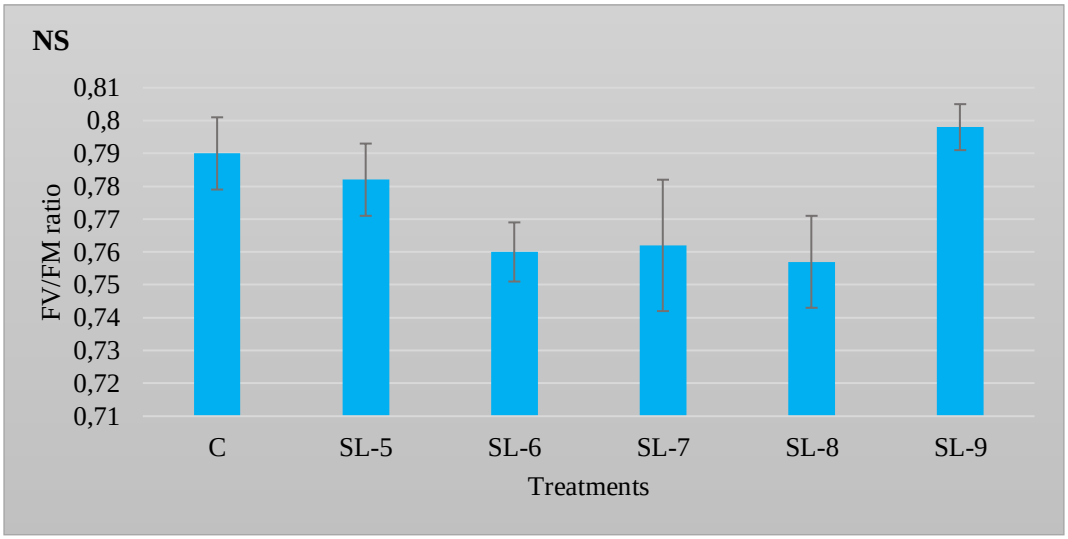


Figure 3.17: FV/FM ratio of C and SL treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no SL application throughout the experiment. Wheat plants sprayed with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) M are labeled on the graph as SL-5, SL -6, SL -7, SL -8, and SL -9 respectively. Blue indicates the mean value of the FV/FM ratio while the. Letters over the bars indicate significant differences between the mean value of the FV/FM ratio of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between SL treated plants in comparison with C, while different letters indicate significant differences between SL treated plants in comparison with the C treatment.*

3.2.1h. The involvement of SL on the number of leaves of phytohormone treatments

SL showed no significant influence on the number of leaves formed by the phytohormone treatments (Figure 3.18).

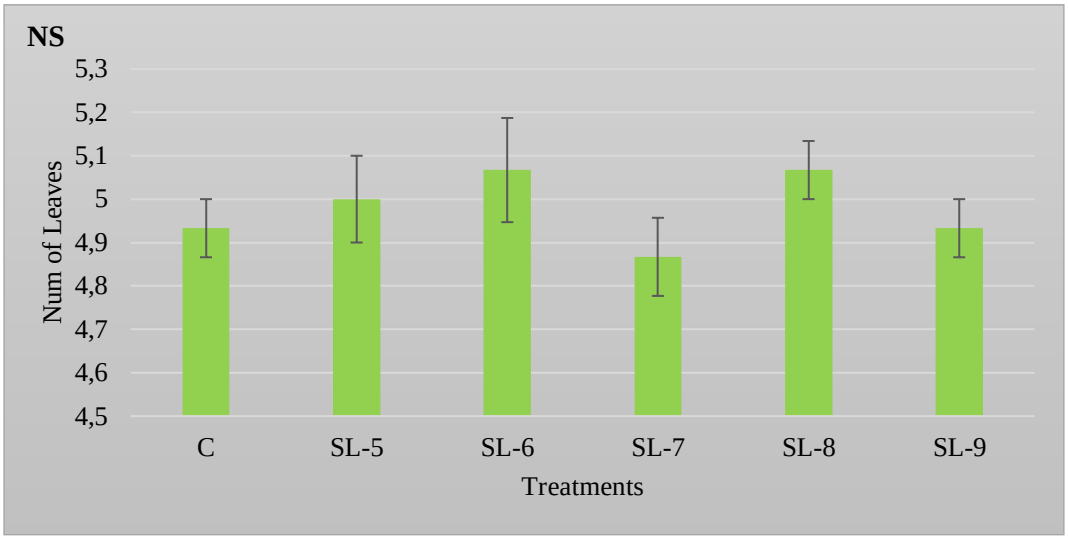


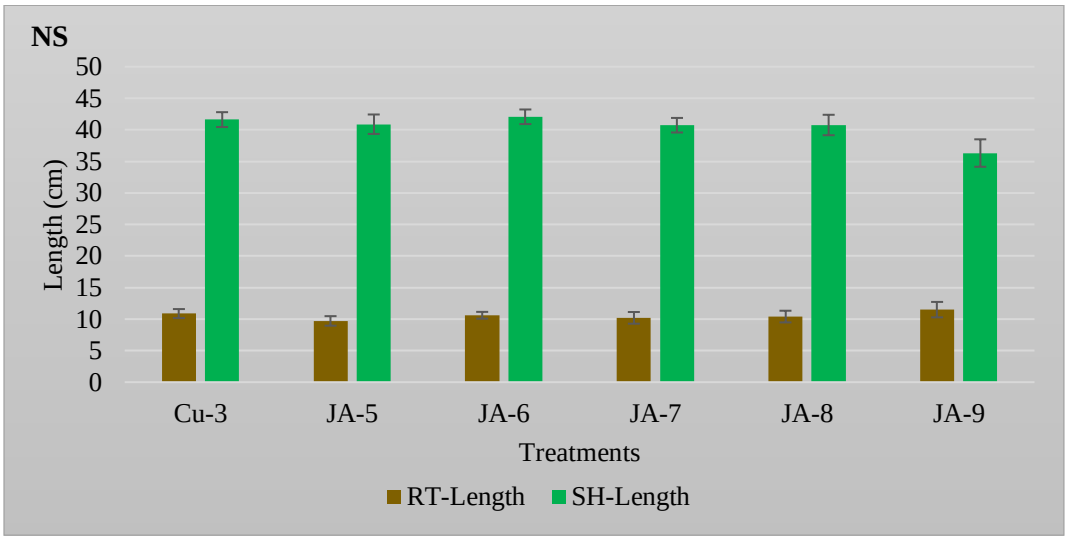
Figure 3.18: The number of leaves of C and SL treated plants in comparison. *Control plants (C) are wheat plants grown in soil containing no HM and no SL application throughout the experiment. Wheat plants sprayed with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations are labeled on the graph as SL-5, SL -6, SL -7, SL -8, and SL -9 respectively. Green bars indicate the mean value of the number of leaves, while the letters above the bars indicate significant differences between the mean value of the number of leaves of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between SL treated plants in comparison with C, while different letters indicate significant differences between SL treated plants in comparison with the C treatment.*

3.2.2. The involvement of JA on the HM stress tolerance on wheat

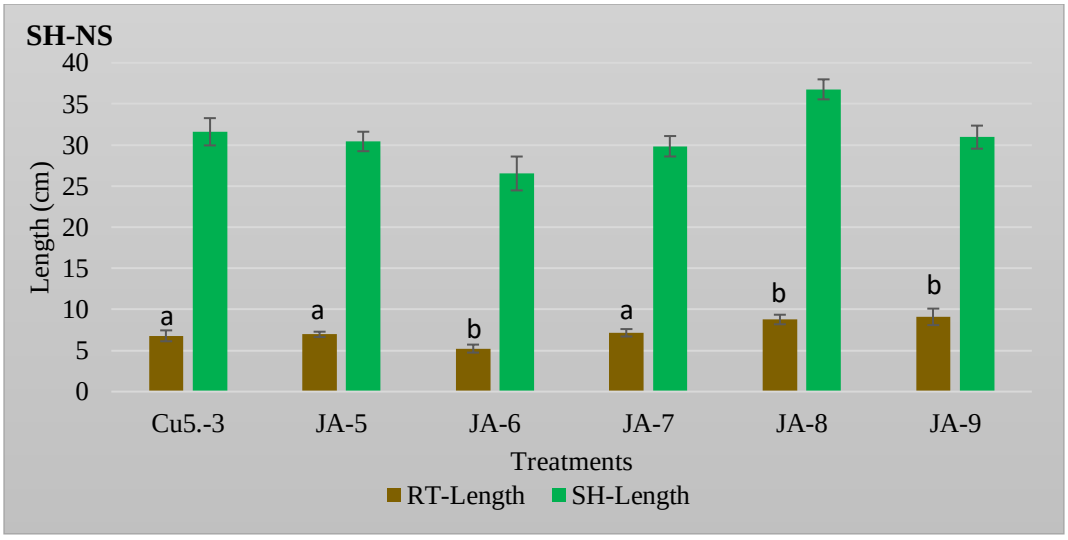
In this study, we used parameters such as the mean values of the root and shoot length, root and shoot weight, FV/FM ratio and number of leaves to investigate the possible involvement of JA on the stress tolerance of wheat to HM induced stress.

3.2.2a. The involvement of JA on root and shoot length of Cu treated plants

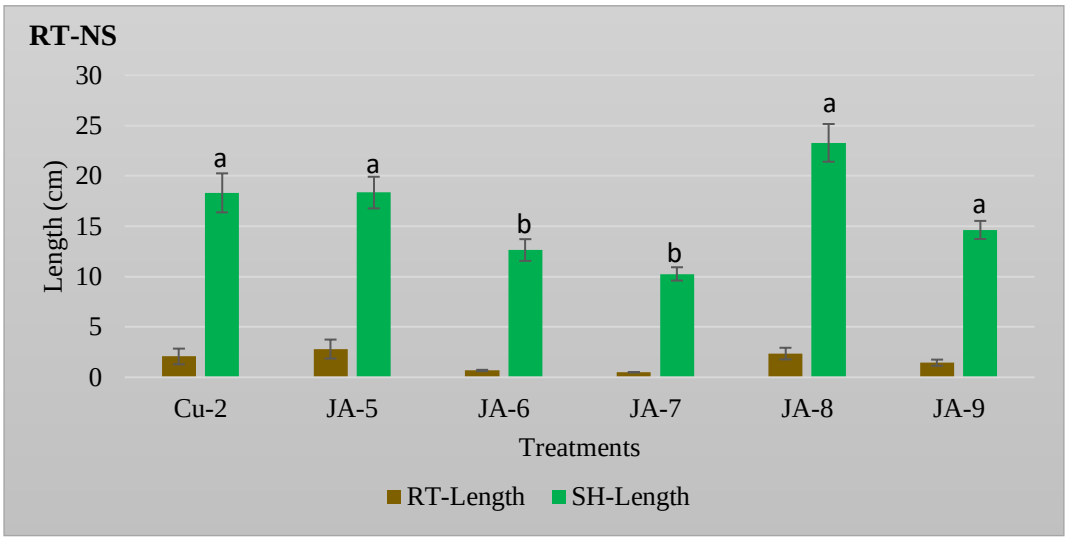
No concentration of JA applied showed any significant influence on the root and shoot length of Cu 10^{-3} M treatments (Figure 3.19a). JA 10^{-6} M was involved in the root length of Cu 5.10^{-3} M treated plants due to the significant decrease in length showed. Still, on the roots of Cu 5.10^{-3} M treatments, JA concentrations of 10^{-8} M and 10^{-9} M significantly increased the root length. On the other hand, the shoots were not significantly influenced by any of the concentrations of JA applied (Figure 3.19b). For the Cu 10^{-2} M treated plants, JA showed no significant influence on the root length but, a significant decrease in the shoot length was showed by the concentrations 10^{-6} M and 10^{-7} M respectively (Figure 3.19c).



a.) Cu 10^{-3} M treated plants in comparison with Cu 10^{-3} /JA treated plants.



b.) Cu 5.10^{-3} M treated plants in comparison with Cu 5.10^{-3} /JA treated plants.

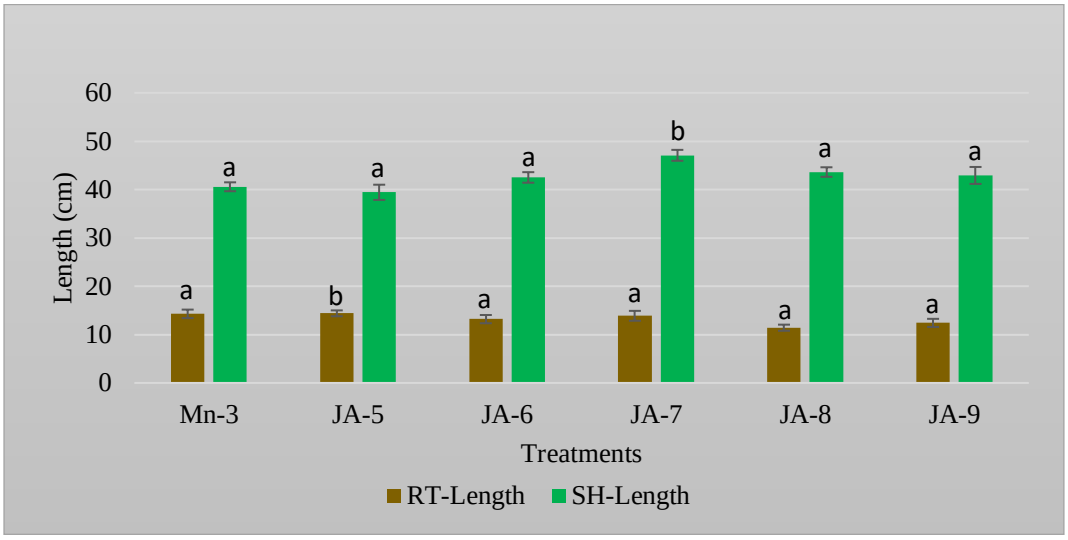


c.) Cu 10^{-2} M treated plants in comparison with Cu 10^{-2} /JA treated plants.

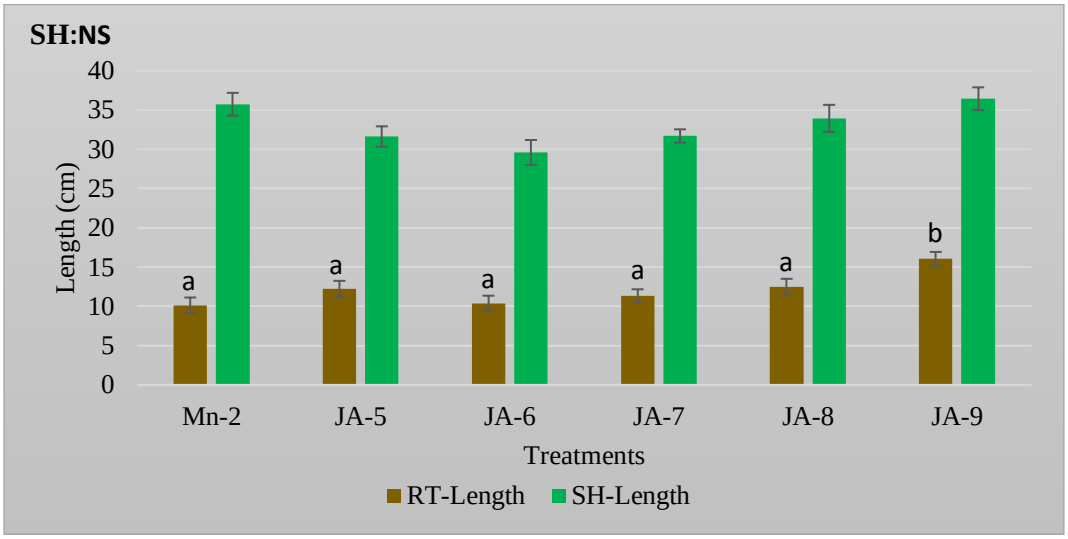
Figure 3.19: Possible involvement of JA on root and shoot length of Cu treated plants. *Control plants (C) are wheat plants grown in soil containing no HM and no JA application throughout the experiment. HM treatments (Cu 10^{-3} (Cu-3), Cu 5.10^{-3}(Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM in soil but not treated with JA acid. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations in combination with the various concentrations of Cu applied to the soil are HM/JA treatments. Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Letters over the bars indicate significant differences between the mean value of the shoot and root length of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments, while different letters indicate significant differences between the HM treatment in comparison with, HM/JA treatments.*

3.2.2b. The involvement of JA on root and shoot length of Mn treated plants

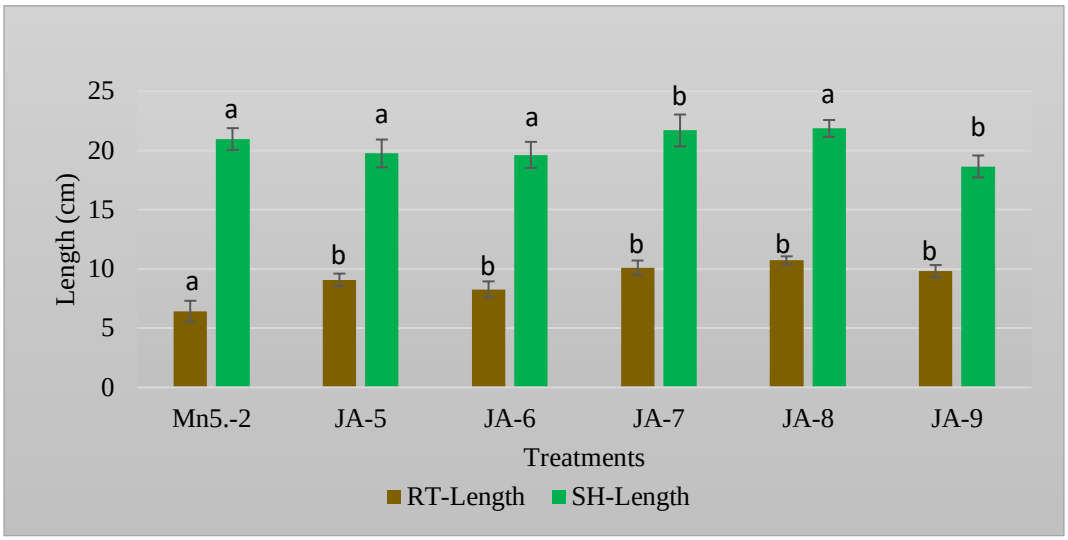
JA 10^{-5} M significantly increased the root length of Mn 10^{-3} M treated plants. On the other hand, JA 10^{-7} M showed a significant increase as well in the shoots (Figure 3.20a). JA 10^{-9} M significantly increased the root length of Mn 10^{-2} M treated plants but on the other hand, showed no significant influence on the shoot length (Figure 3.20b). All the respective concentrations of JA applied showed significant increases in the root length of Mn 5.10^{-2} M treated plants. For the shoots, JA 10^{-7} M showed a significant increase in the shoot length, while JA 10^{-9} M showed a significant decrease in the shoot length of Mn 5.10^{-2} M treated plants respectively (Figure 3.20c).



a.) Mn 10^{-3} M treated plants in comparison with Mn 10^{-3}/JA treated plants.



b.) Mn 10^{-2} M treated plants in comparison with Mn 10^{-2}/JA treated plants.

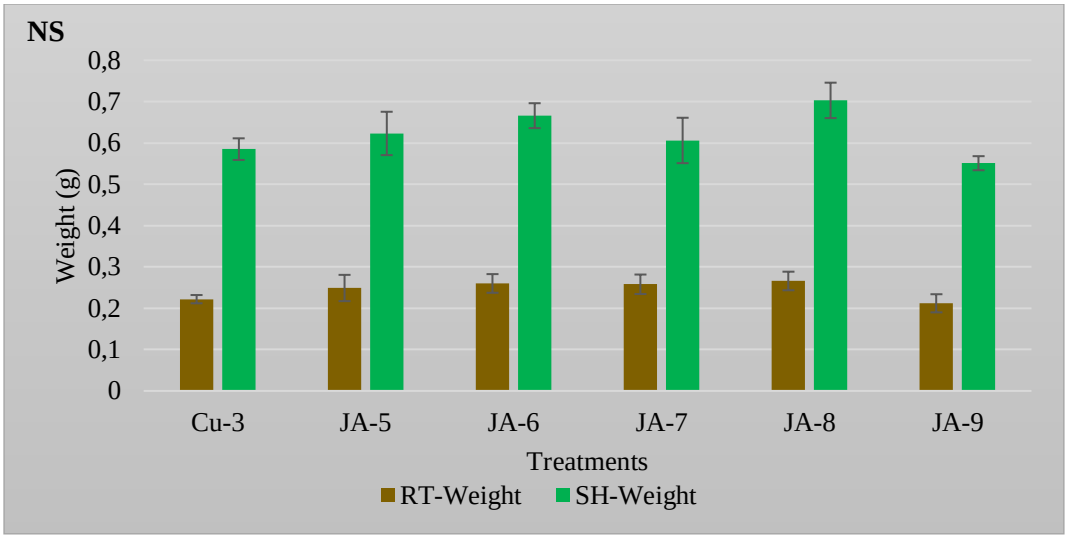


c.) Mn 5.10^{-2} M treated plants in comparison with Mn 5.10^{-2}/JA treated plants.

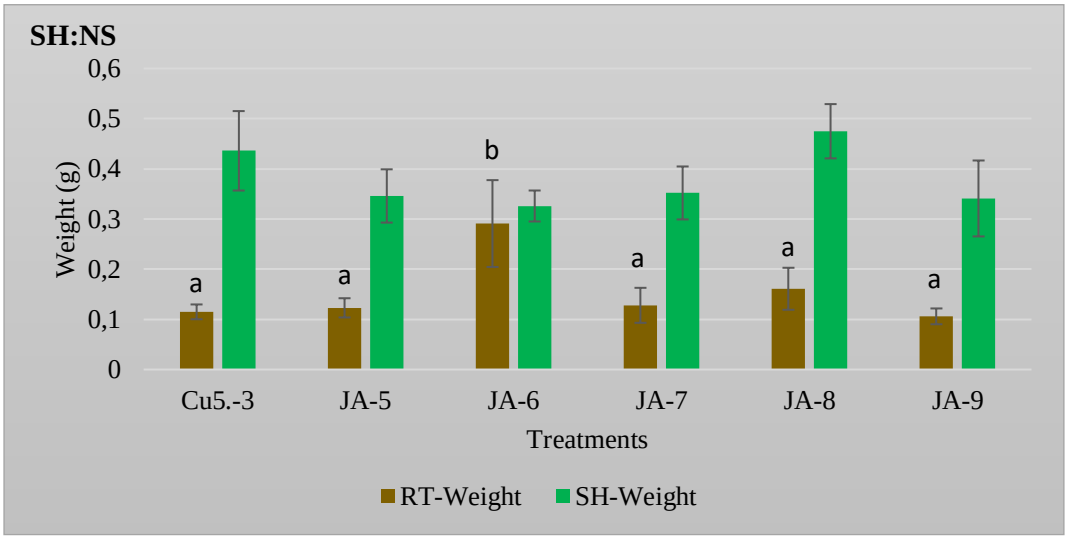
***Figure 3.20**: **Possible involvement of JA on root and shoot length of Mn treated plants.** HM treatments ((Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn $10^{5(-2)}$ (Mn5.-2)) M, are wheat plants exposed to HM in soil but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various concentrations of Cu applied to the soil are HM/JA treatments. Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Letters over the bars indicate significant differences between the mean value of the shoot and root length of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments, while different letters indicate significant differences between the HM treatment in comparison with, HM/JA treatments.*

3.2.2c. Possible involvement of JA on root and shoot weight of Cu treated plants

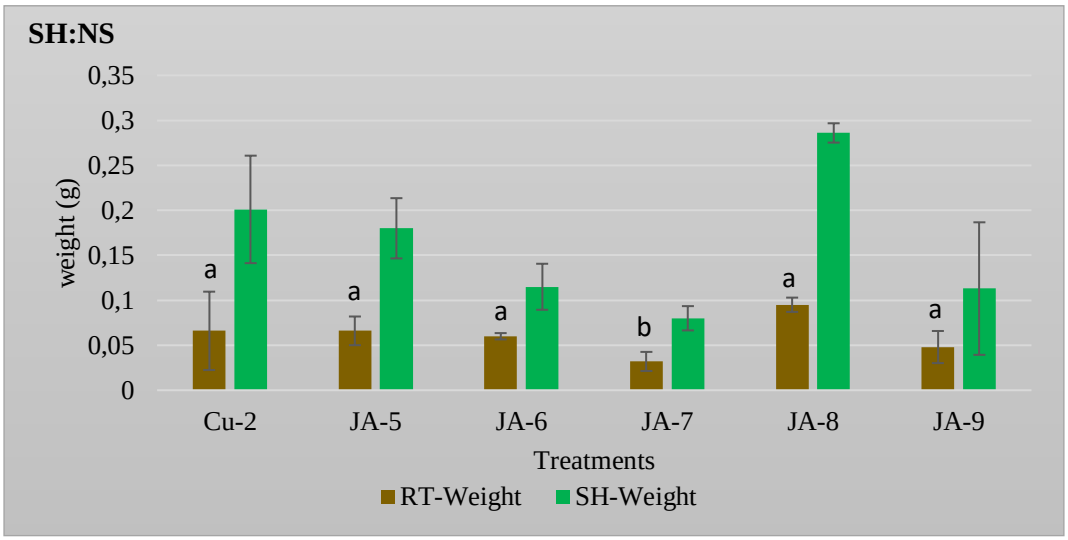
JA showed no significant influence on the roots and the shoot biomass of the Cu 10^{-3} M treatments (Figure 3.21a). JA 10^{-6} M significantly increased the shoot weight of Cu 5.10^{-3} M treatments, meanwhile, JA showed no influence on the shoot weight (Figure 3.21b). For the Cu 10^{-2} M treatments, JA 10^{-7} M showed a significant decrease in the root weight, while none of the other concentrations showed any influence on the shoot weight (Figure 3.21c).



a.) Cu 10^{-3} M treated plants in comparison with Cu 10^{-3}/JA treated plants.



b.) Cu 5.10^{-3} M treated plants in comparison with Cu 5.10^{-3}/JA treated plants.

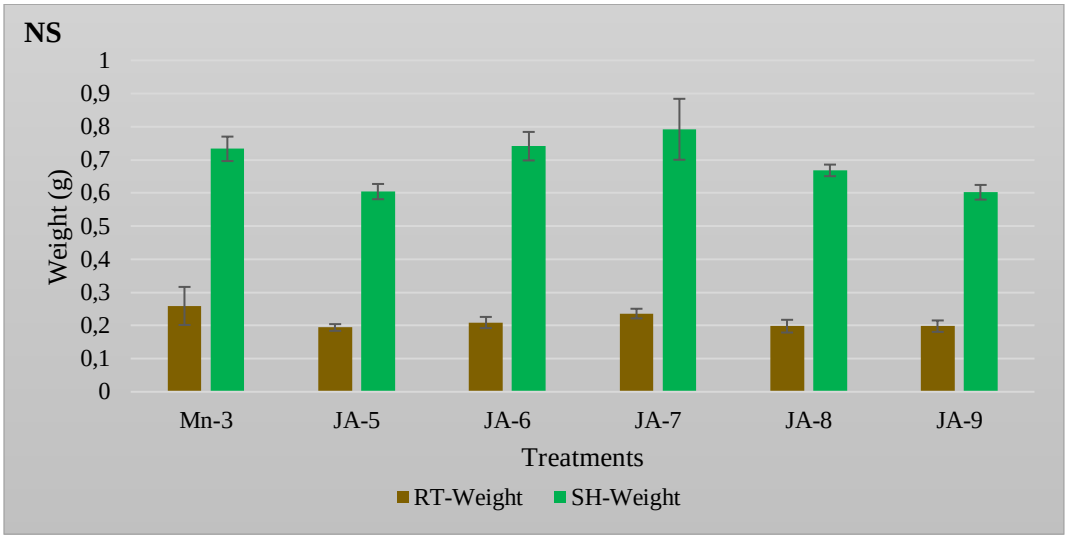


c) Cu 10^{-2} M treated plants in comparison with Cu 10^{-2}/JA treated plants.

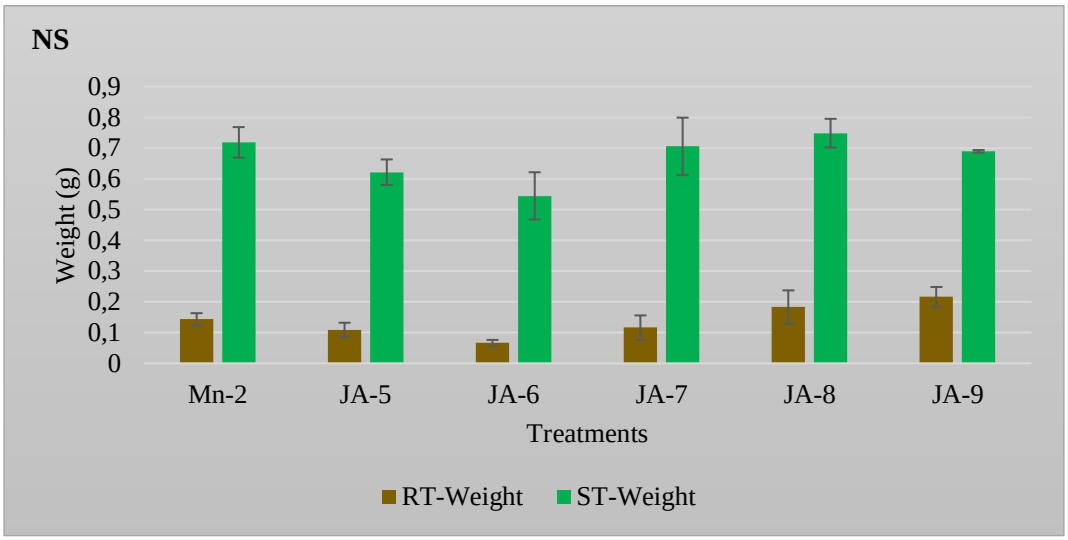
***Figure 3.21**: **Possible involvement of JA on root and shoot weight of Cu treated plants.** HM treated plants (Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various HM in soil respectively are considered HM/JA treatments. Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Letters above the bars indicate significant differences between the mean value of the shoot and root weights of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/JA treatments.*

3.2.2d. Possible involvement of JA on root and shoot weight of Mn treated plants

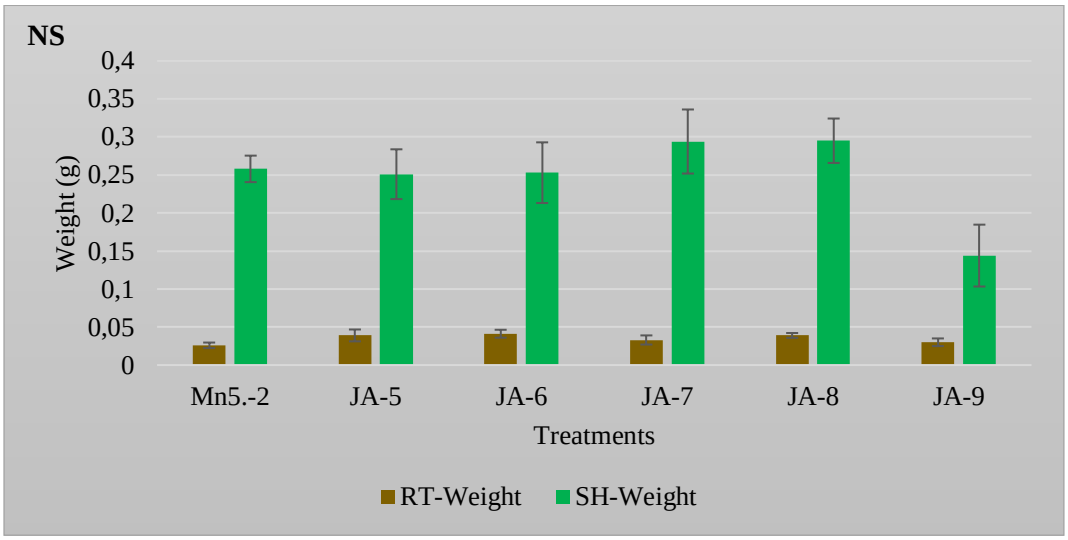
For the plants treated with Mn 10^{-3} M, Mn 10^{-2} M and Mn 5.10^{-2} M, JA of all concentrations showed no significant influence on the roots and shoot weight of the plants (Figure 3.22a, b, and c).



a.) Mn 10^{-3} M treated plants in comparison with Mn 10^{-3}/JA treated plants.



b.) Mn 10^{-2} M treated plants in comparison with Mn 10^{-2}/JA treated plants.

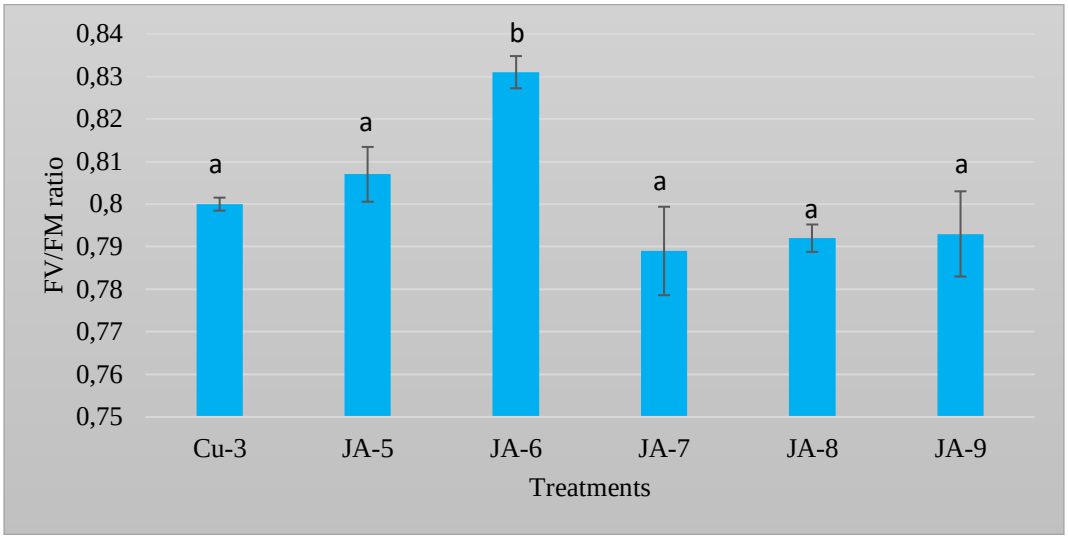


c.) Mn 5.10^{-2} M treated plants in comparison with Mn 5.10^{-2}/JA treated plants.

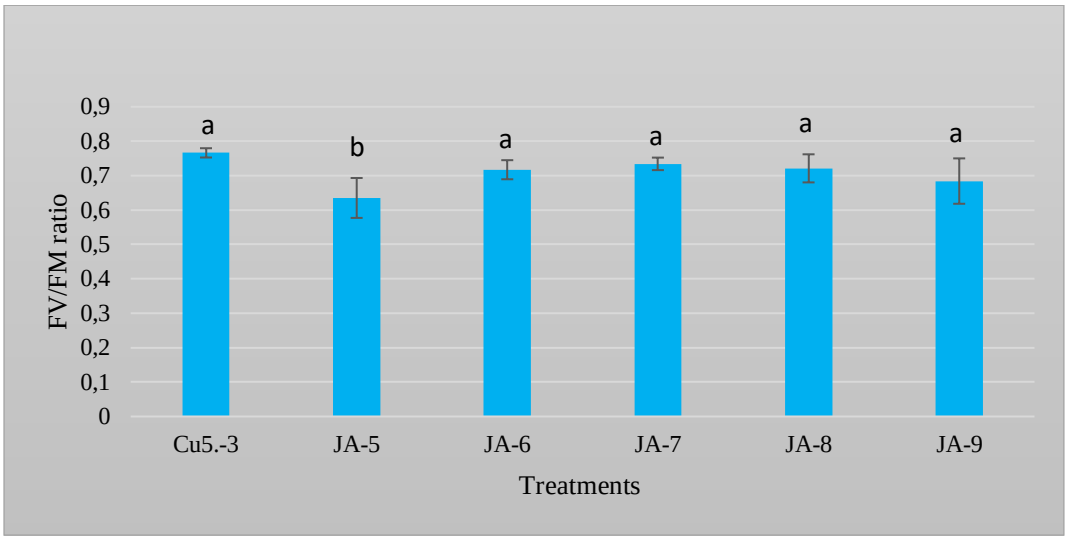
***Figure 3.22**: **Possible involvement of JA on root and shoot weight of Mn treated plants.** HM treatments (Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5(-2)) M, are wheat plants exposed to HM but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various HM in soil respectively are considered HM/JA treatments. Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Letters above the bars indicate significant differences between the mean value of the shoot and root weights of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/JA treatments.*

3.2.2e. Possible involvement of JA on the FV/FM ratio of Cu treated plants

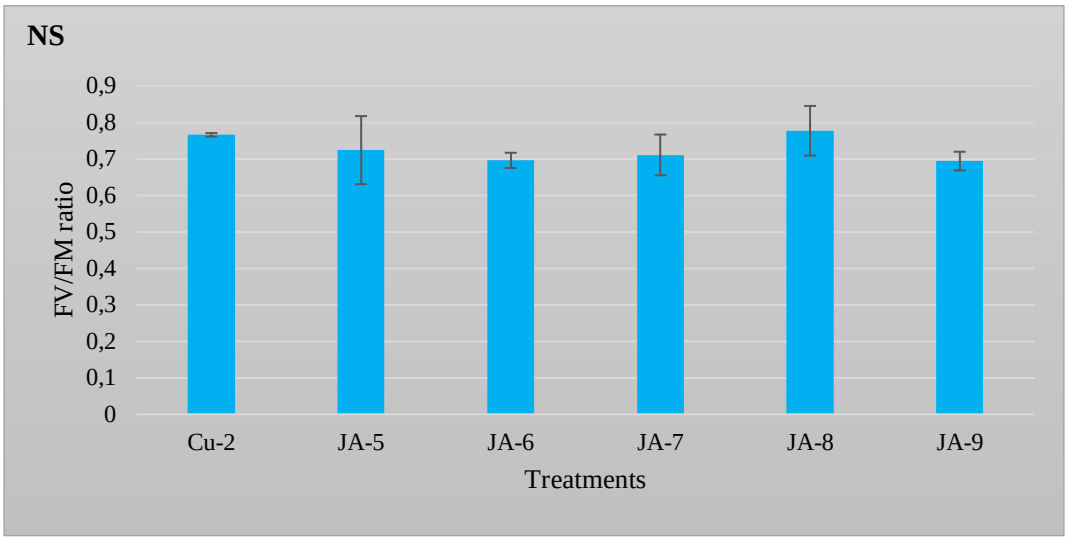
JA 10^{-6} M significantly increased the FV/FM ratio of the leaves of Cu 10^{-3} M treatments (Figure 3.23a). JA 10^{-5} M was significantly reduced the FV/FM ratio of the leaves of the Cu 5.10^{-3} M treatments (Figure 3.23b). No significant influence of JA was shown by the plants treated with Cu 10^{-2} (Figure 3.23c).



a.) Cu 10^{-3} M treated plants in comparison with Cu 10^{-3}/JA treated plants.



b.) Cu 5.10^{-3} M treated plants in comparison with Cu 5.10^{-3}/JA treated plants.

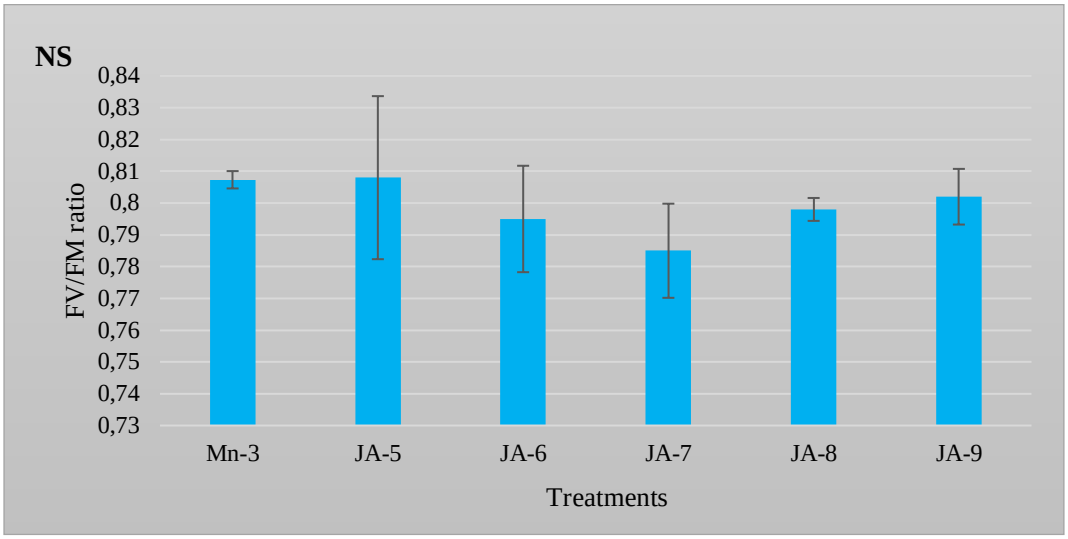


(c) Cu 10^{-2} M treated plants in comparison with Cu 10^{-2}/JA treated plants.

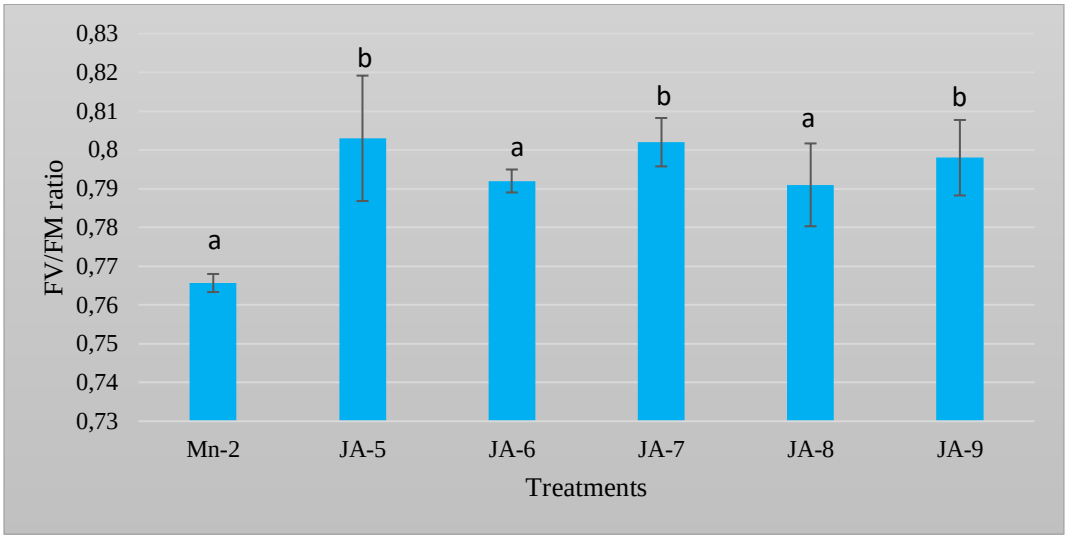
***Figure 3.23**: **Possible involvement of JA on the FV/FM ratio of Cu treated plants.** HM treated plants Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) M, in combination with the various HM in the soil are considered HM/JA treatments. Blue bars indicate the mean value of the FV/FM ratio. Letters above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between HM treatments in comparison with HM/JA treatments while different letters indicate significant differences between HM treatments in comparison with, HM/JA treatments.*

3.2.2f. Possible involvement of JA on the FV/FM ratio of Mn treated plants

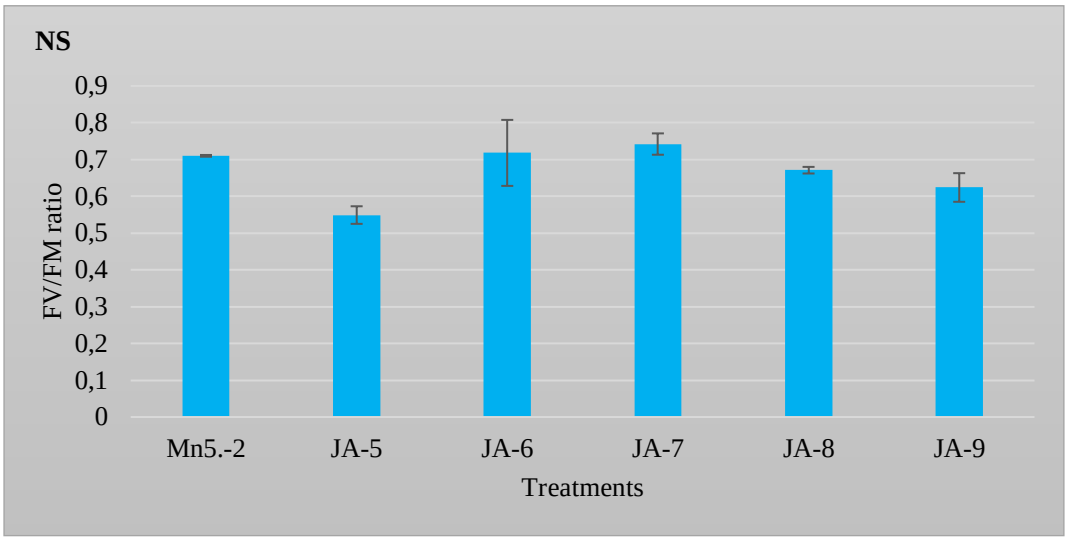
JA showed no significant influence on the FV/FM ratio of Mn 10^{-3} M and Mn 5.10^{-2} M treatments (Figure 3.24a and Figure 3.24b). Meanwhile, for the Mn 10^{-2} M treatments, JA 10^{-5} M, 10^{-7} M, and 10^{-9} M both significantly increased the FV/FM ratio of the plant leaves (Figure 3.24c)



a.) Mn 10^{-3} M treated plants in comparison with Mn 10^{-3}/JA treated plants.



b.) Mn 10^{-2} M treated plants in comparison with Mn 10^{-2}/JA treated plants.

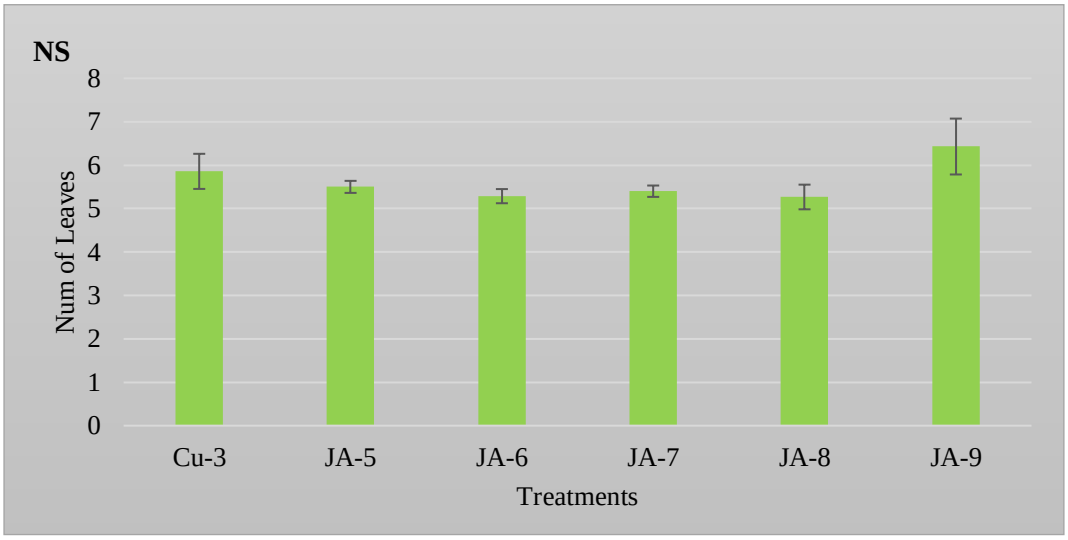


c.) Mn 5.10^{-2} M treated plants in comparison with Mn 5.10^{-2} /JA treated plants.

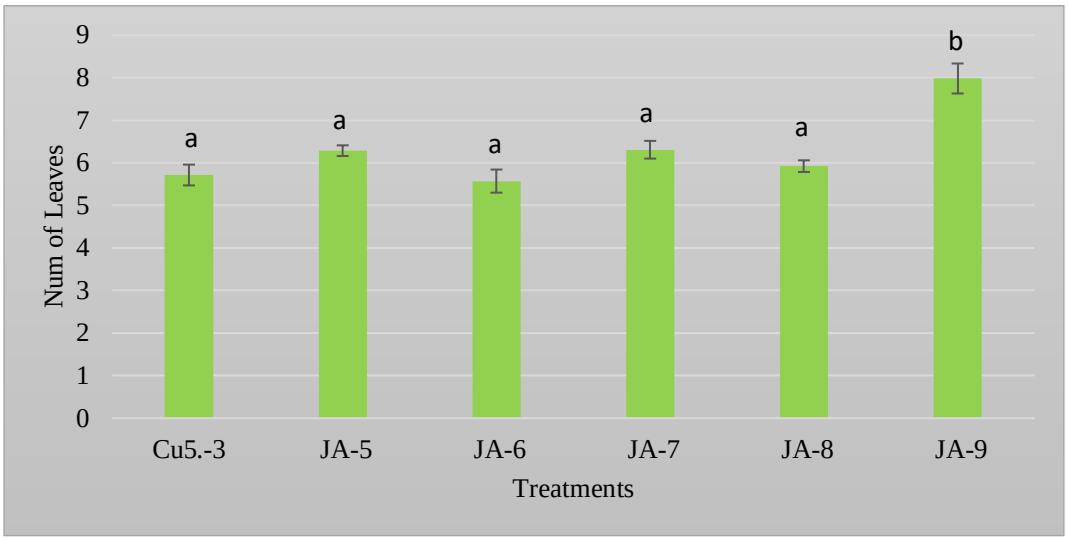
***Figure 3.24**: **Possible involvement of JA on the FV/FM ratio of Mn treated plants.** HM treated plants ((Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2)) M, are wheat plants exposed to HM but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) M in combination with the various HM in the soil are considered HM/JA treatments. Blue bars indicate the mean value of the FV/FM ratio. Letters above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments while different letters indicate significant differences between the HM treatment in comparison with the HM/JA treatments.*

3.2.2g. Possible involvement of JA on the number of leaves formed by Cu treated plants

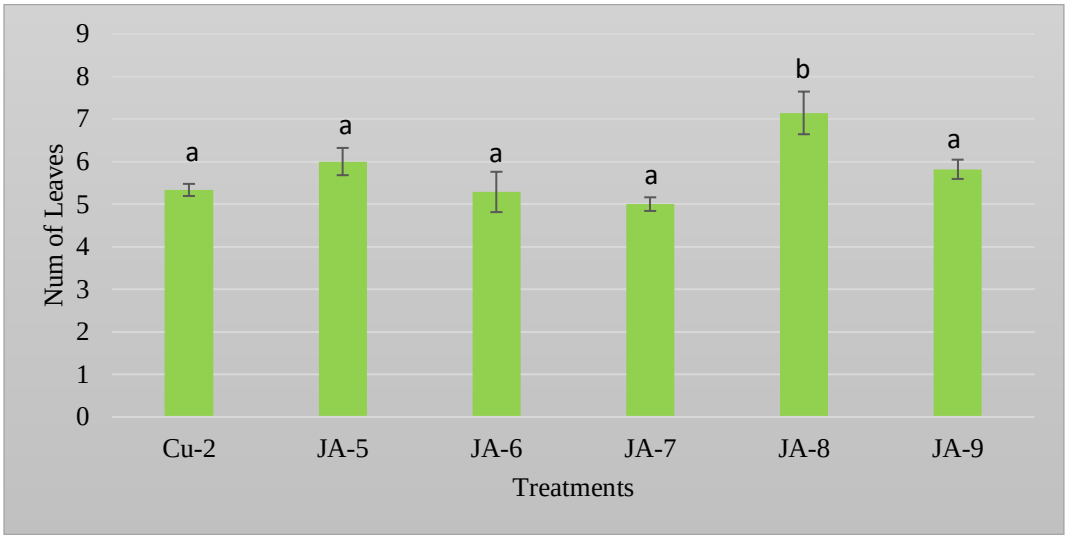
JA showed no significant influence on the number of leaves formed by the plants treated with Cu 10^{-3} M (Figure 3.25a). JA 10^{-9} M significantly increased the number of leaves formed by Cu 5.10^{-3} M treated plants (Figure 3.25b). JA 10^{-8} M significantly increased the number of leaves formed by Cu 10^{-2} M treated plants (Figure 3.25c).



a.) Cu 10^{-3} M treated plants in comparison with Cu 10^{-3}/JA treated plants.



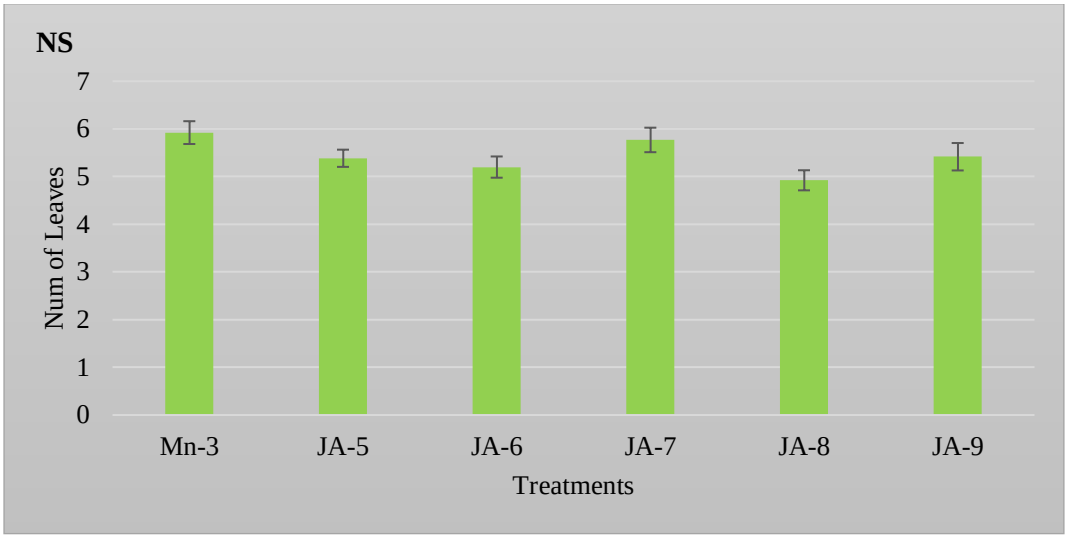
b.) Cu 5.10^{-3} M treated plants in comparison with Cu 5.10^{-3}/JA treated plants.



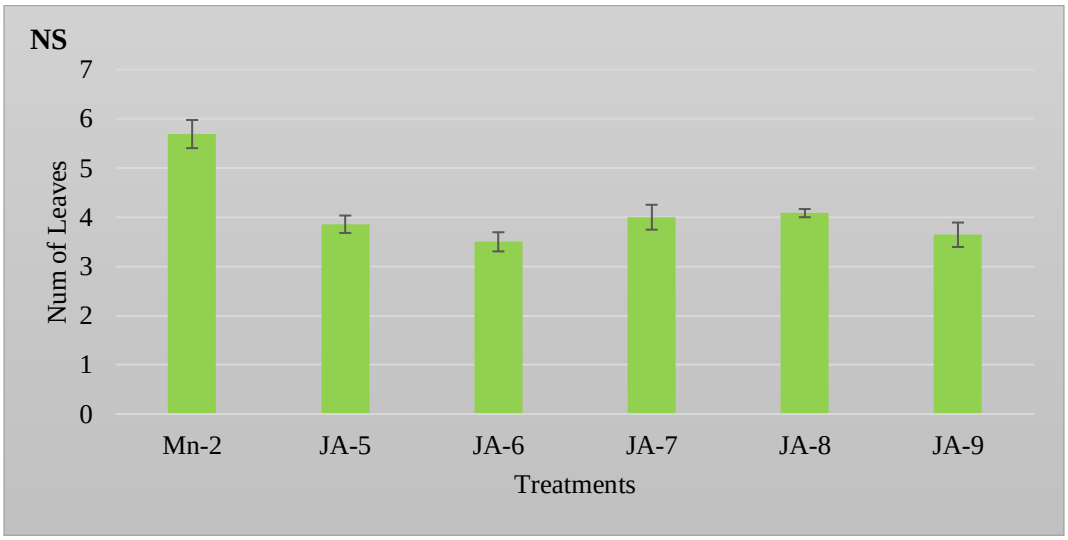
c.) Cu 10^{-2} M treated plants in comparison with Cu 10^{-2}/JA treated plants.

***Figure 3.25**:* **Possible involvement of JA on the number of leaves formed by Cu treated plants.** *HM treated plants (Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3 and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM in soil but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations respectively, in combination with the various HM applied to the soil are considered HM/JA treatments. Green bars indicate the mean value of the number of leaves. Letters above the bars indicate significant differences between the mean value of the number of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/JA treatments.*

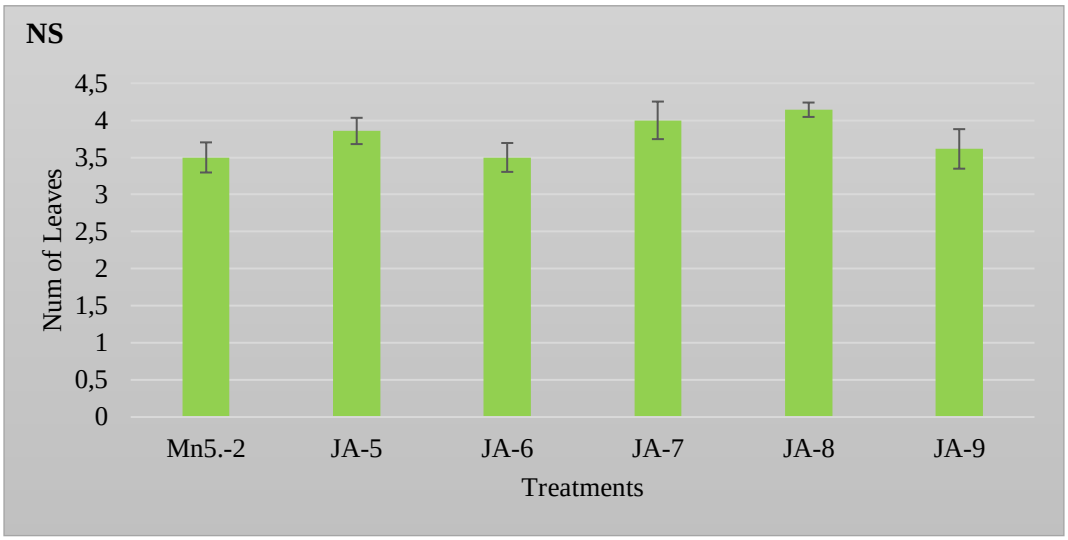
3.2.2h. Possible involvement of JA on the number of leaves formed by Mn treated plants
All the concentrations of JA tested showed no significant influence on the number of leaves formed by the different concentrations of Mn treatments (Figure 3.26a, b, and c).



a.) Mn 10^{-3} M treated plants in comparison with Mn 10^{-3}/JA treated plants.



b.) Mn 10^{-2} M treated plants in comparison with Mn 10^{-2}/JA treated plants.



(c) Mn 5.10^{-2} M treated plants in comparison with Mn 5.10^{-2}/JA treated plants.

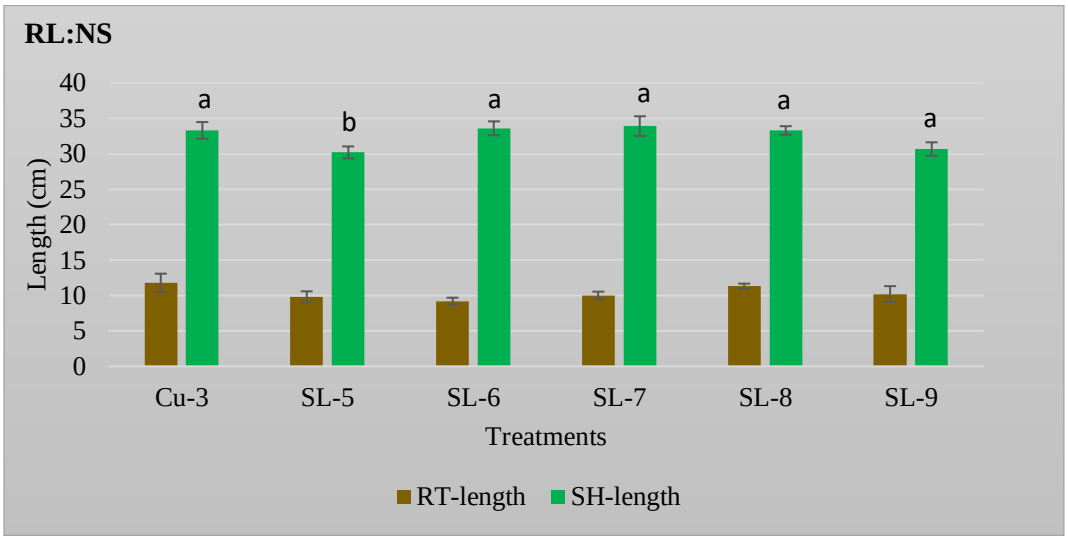
***Figure 3.26**: **Possible involvement of JA on the number of leaves formed by Mn treated plants.** HM treated plants (Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5(-2)) M, are wheat plants exposed to HM but not treated with JA. Wheat plants treated with JA (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations respectively, in combination with the respective HM applied to the soil are considered HM/JA treatments. Green bars indicate the mean value of the number of leaves. Letters above the bars indicate significant differences between the mean value of the number of leaves of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/JA treatments, while different letters indicate significant differences between the HM treatment in comparison with, HM/JA treatments.*

3.2.3. Involvement of SL in HM induced stress on wheat

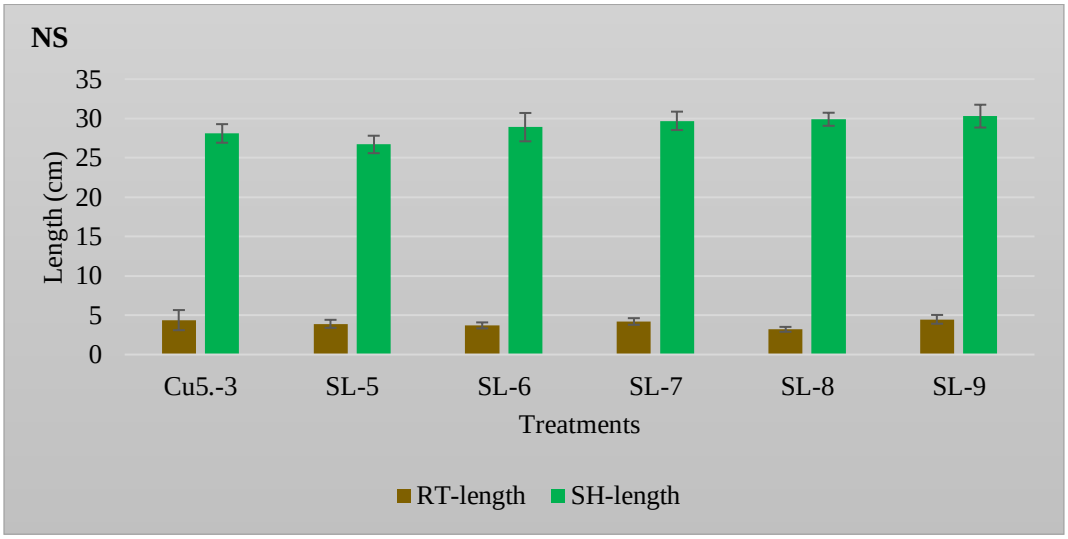
In this study, the possible involvement of SL on HM induced stress on wheat was investigated. With suggestions from previous studies that SL is involved in causing structural changes to the root, shoot and leaves in plants exposed to stress conditions, this experiment was designed around investigating the possible involvement of SL on parameters such as root and shoot length, root and shoot weight, FV/FM ratio of leaves and number of leaves in plants which were stress induced by HM stress.

3.2.3a. Possible involvement of SL on root and shoot length of Cu treated plants

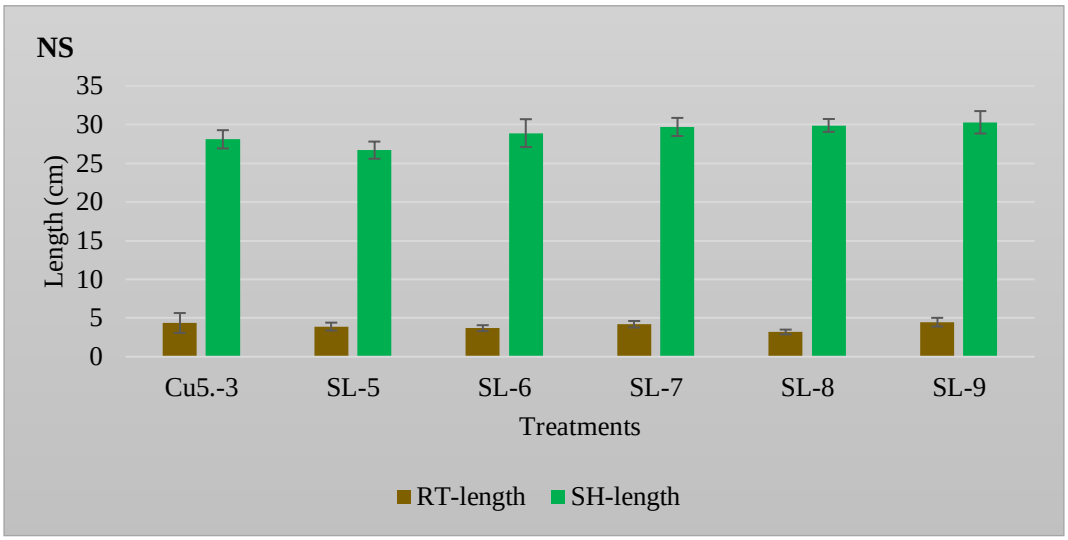
SL 10^{-5} M significantly decreased the shoot length of Cu 10^{-3} M treated plants, meanwhile, there was no significant involvement of SL on the root length (Figure 3.27a). On the other hand, there was no significant involvement of all concentrations of SL applied, on the root and shoot length of Cu 5.10^{-3} M and Cu 10^{-2} M treatments respectively (Figure 3.27b and **c**).



a.) Effects of SL on root and shoot length of Cu 10^{-3} M treated plants.



b.) Effects of SL on root and shoot weight of Cu 5.10^{-3} M treated plants.

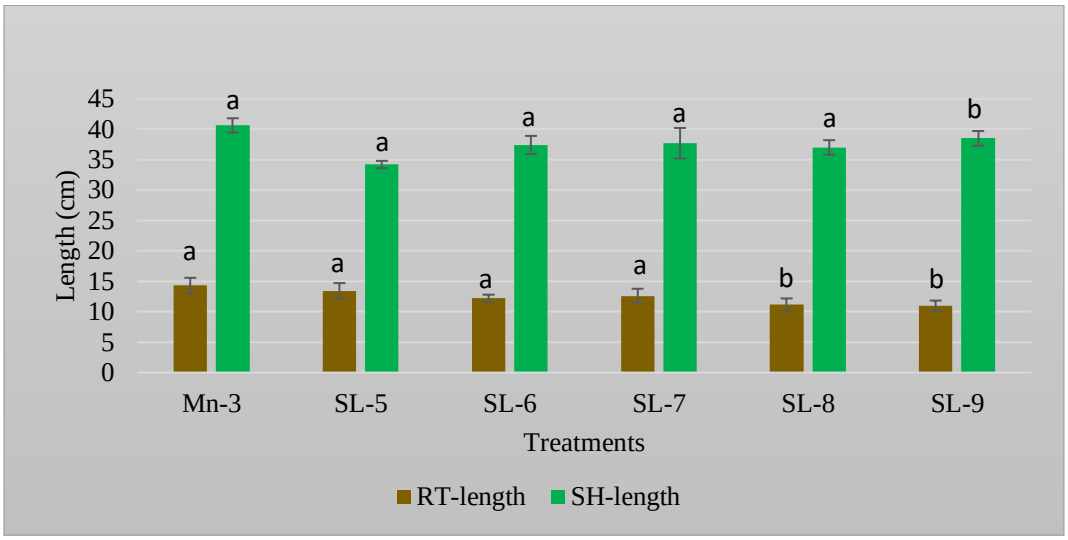


c.) Effects of SL on root and shoot weight of Cu 10^{-2} M treated plants.

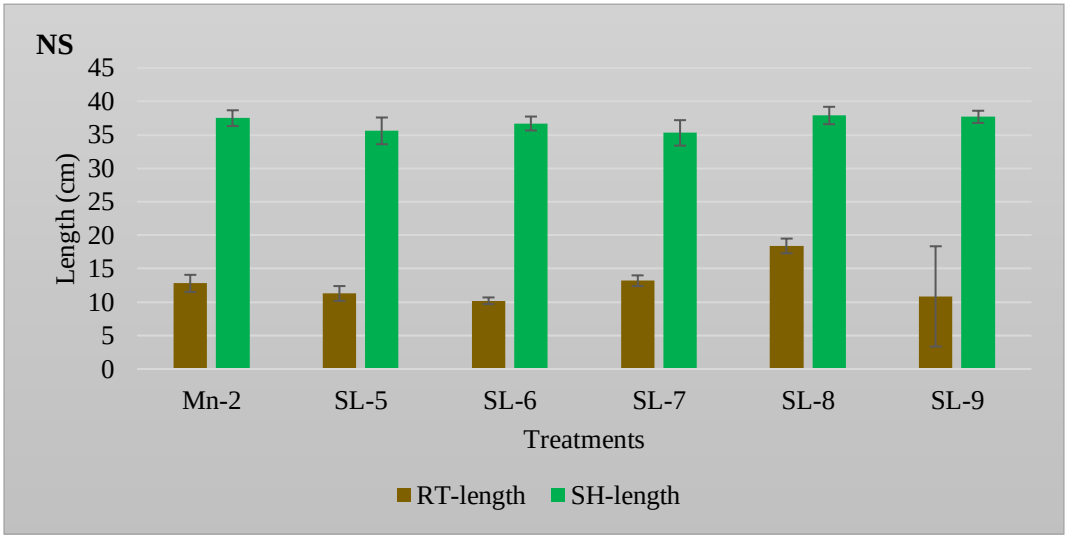
***Figure 3.27**: **Possible involvement of SL on root and shoot length of Cu treated plants**. HM treated plants (Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL, (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various HM, applied to the soil, are considered HM/SL treatments. Brown bars show the mean value of the root length while green bars show the mean value of the shoot length. Letters above the bars indicate significant differences between the mean value of the root and shoot length of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment, in comparison with HM/SL treatments, while different letters indicate significant differences between the HM treatment in comparison with the HM/SL treatments.*

3.2.3b. Possible involvement of SL on the root and shoot length of Mn treated plants

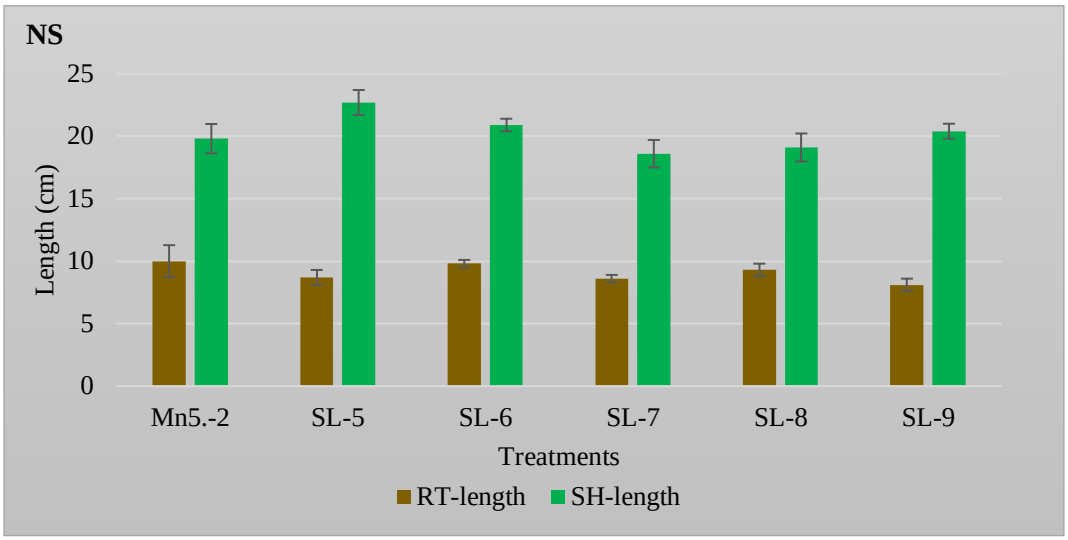
SL 10^{-8} M and 10^{-9} M significantly decreased the root length of Mn 10^{-3} M treated plants respectively. on the other hand, SL 10^{-9} M significantly decreased the shoot length of Mn 10^{-3} M treated plants (Figure 3.28a). On the other hand, there was no significant influence of all concentrations of SL applied, on the root and shoot length of Mn 10^{-2} M and Mn 5.10^{-2} M treatments respectively (Figure 3.28b and c).



a.) Effects of SL on root and shoot length of Mn 10^{-3} M treated plants.



b.) Effects of SL on root and shoot length of Mn 10^{-2} M treated plants.

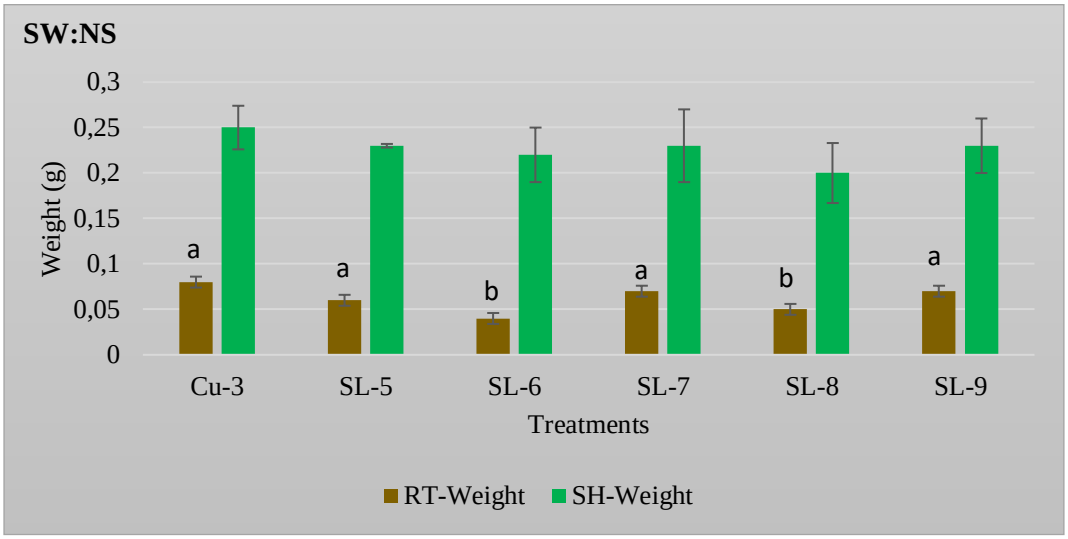


c.) Possible influence of SL on root and shoot length of Mn 5.10^{-2} M treated plants.

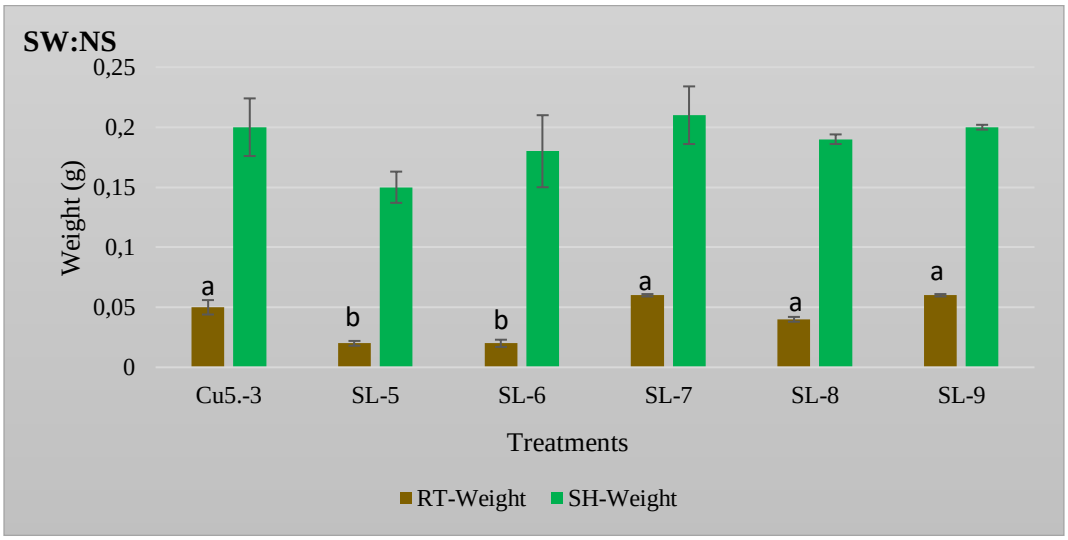
FIGURE 3.28: Possible involvement of SL on the root and shoot length of Mn treated plants. *HM treated plants (Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL, (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various HM, applied to the soil, are considered HM/SL treatments. Brown bars show the mean value of the root length while green bars show the mean value of the shoot length. Letters above the bars indicate significant differences between the mean value of the root and shoot length of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment, in comparison with HM/SL treatments, while different letters indicate significant differences between the HM treatment in comparison with the HM/SL treatments.*

3.2.3c. Possible involvement of SL on root and shoot weight of Cu treated plants

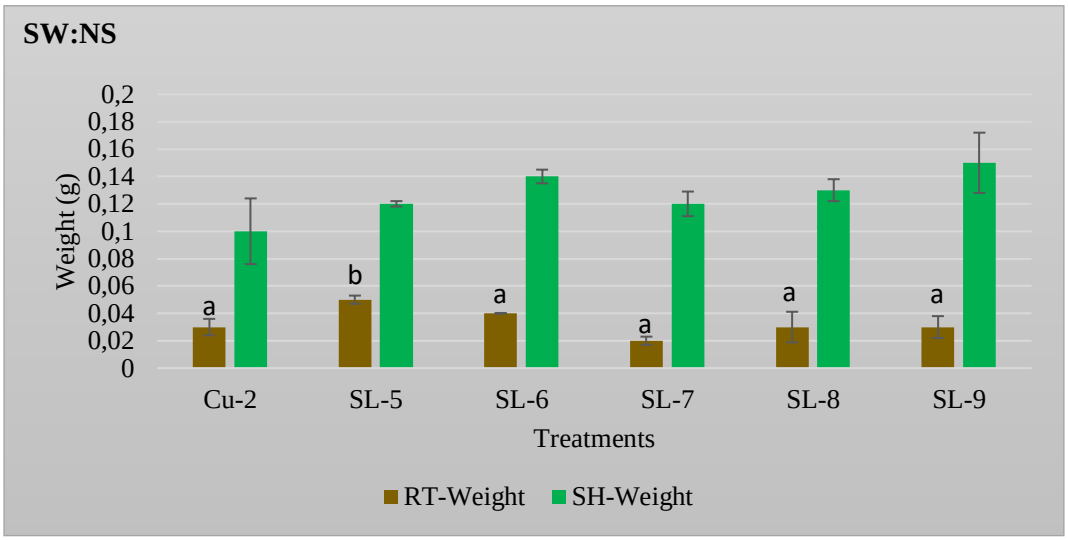
SL 10^{-6} M and SL 10^{-8} M both significantly decreased the root weight of Cu 10^{-3} M treated plants. on the other hand, all concentrations of SL applied showed no significant influence on the shoot weight of Cu 10^{-3} M treated plants (Figure 3.29a). SL 10^{-5} M and SL 10^{-6} M both significantly decreased the root weight of Cu 5.10^{-3} M treated plants. On the other hand, all concentrations of SL applied showed no significant influence on the shoot weight of Cu 5.10^{-3} M treated plants (Figure 3.29b). SL 10^{-5} M significantly increased the root weight of Cu 10^{-2} M treated plants. on the other hand, all concentrations of SL applied showed no significant influence on the shoot weight of Cu 10^{-2} M treated plants (Figure 3.29c).



a.) Effects of SL on root and shoot weight of Cu 10^{-3} M treated plants**.**



b.) Effects of SL on root and shoot weight of Cu 5.10^{-3} M treated plants**.**

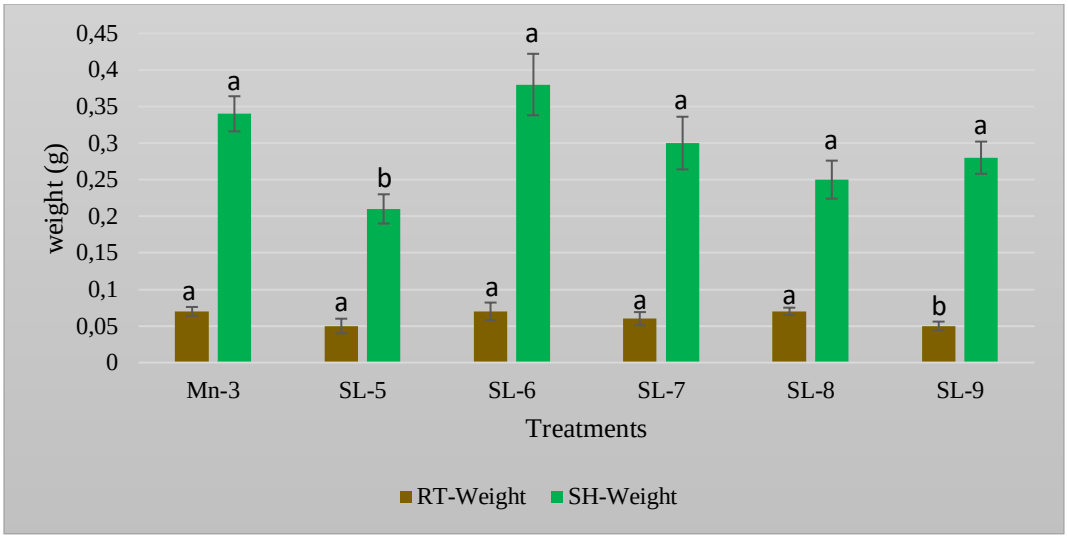


c.) Effects of SL on root and shoot weight of Cu 10^{-2} M treated plants.

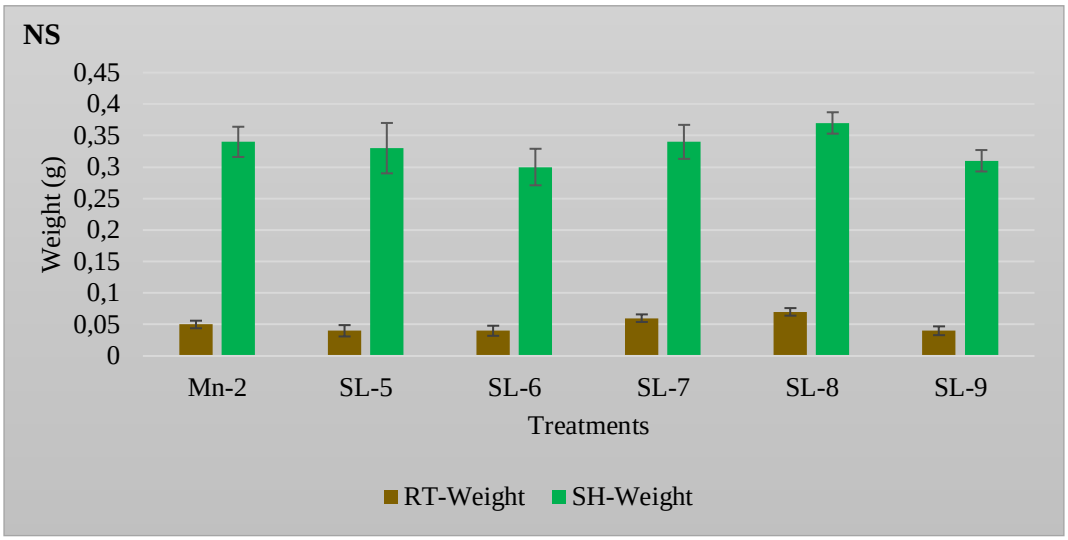
***Figure 3.29*: Possible involvement of SL on root and shoot weight of Cu treated plants.** *HM treated plants (Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL, (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various HM, applied to the soil respectively, are considered HM/SL treatments. Brown bars show the mean value of the root weight while green bars show the mean value of the shoot weight. Letters above the bars indicate significant differences between the mean value of the root and shoot weight of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment, in comparison with HM/SL treatments, while different letters indicate significant differences between the HM treatment in comparison with the HM/SL treatments.*

3.2.3d. Possible involvement of SL on root and shoot weight of Mn treated plants

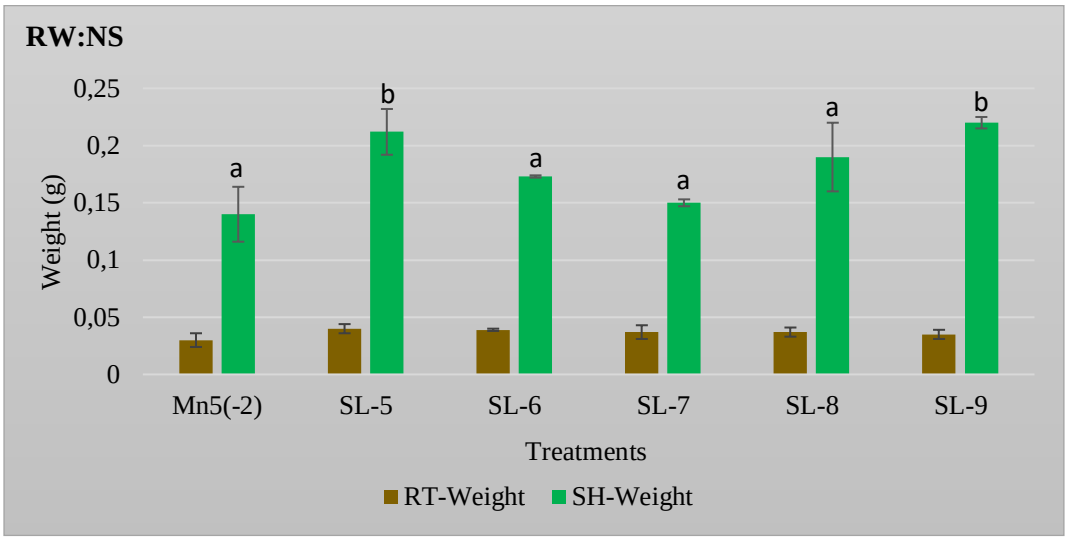
SL 10^{-9} M significantly decreased the root weight, while SL 10^{-5} M significantly decreased the shoot weight of Mn 10^{-3} M treated plants respectively (Figure 3.30a). No significant influence was shown by all concentrations of SL applied, on the root and shoot weight of Mn 10^{-2} M treated plants (Figure 3.30b). SL 10^{-5} M and SL 10^{-9} M significantly increased the shoot weight of Mn 5.10^{-2} M treated plants respectively Figure 3.30c.



a.) Effects of SL on root and shoot weight of Mn 10^{-3} M treated plants.



b.) Effects of SL on root and shoot weight of Mn 10^{-2} M treated plants.

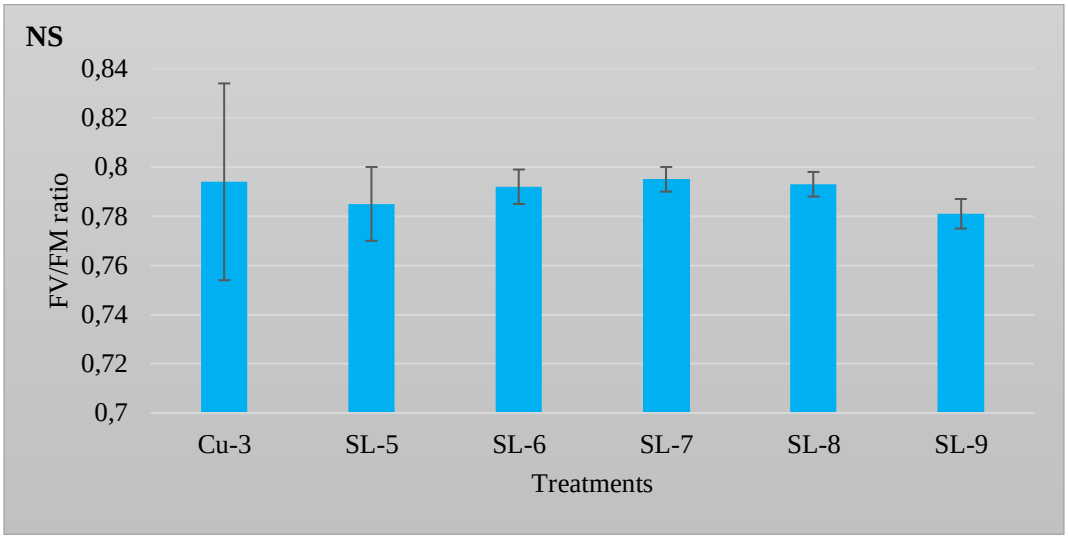


c.) Effects of SL on root and shoot weight of Mn 5.10^{-3} M treated plants.

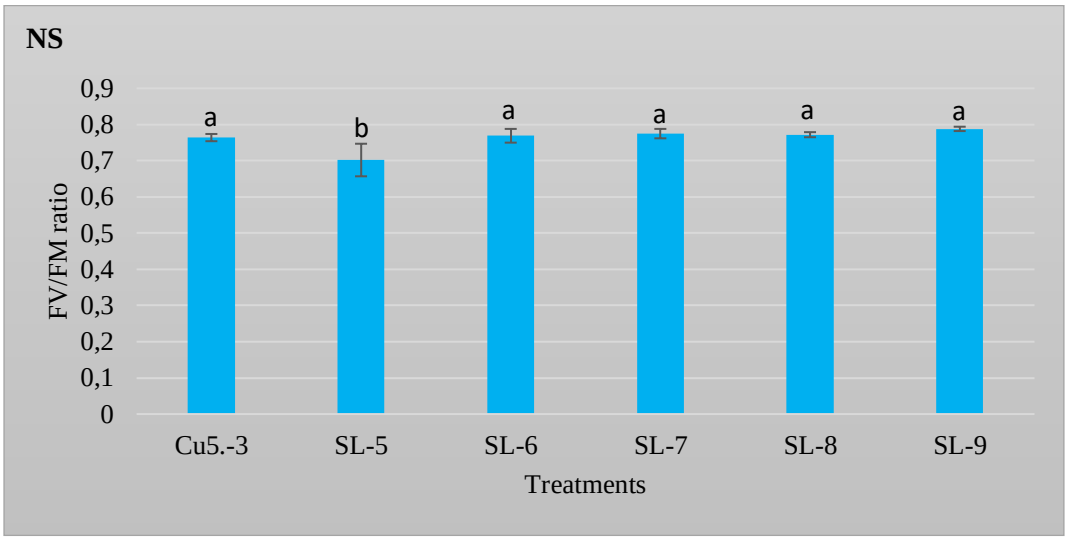
Figure 3.30: Possible involvement of SL on root and shoot weight of Mn treated plants. *HM treated plants (Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL, (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations, in combination with the various HM, applied to the soil, are considered HM/SL treatments. Brown bars show the mean value of the root weight while green bars show the mean value of the shoot weight. Letters above the bars indicate significant differences between the mean value of the root and shoot weight of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment, in comparison with HM/SL treatments, while different letters indicate significant differences between the HM treatment in comparison with the HM/SL treatments.*

3.2.3e. Possible involvement of SL on the FV/FM ratio of Cu treated plants

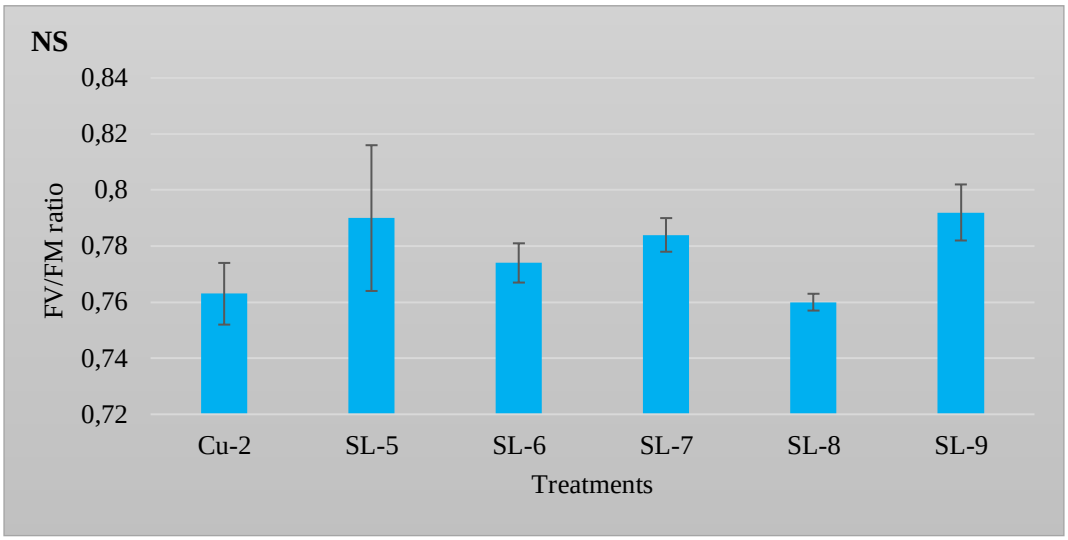
All the concentrations of SL applied on the Cu 10^{-3} M and Cu 10^{-2} M treated plants showed no significant influence on the FV/FM ratio of the respective plant leaves (Figure 3.31a and c). SL10^{-5} M significantly decreased the FV/FM ratio of the Cu 5.10^{-3} M treated plants (Figure 3.31b).



a.) Effects of SL on FV/FM ratio of Cu 10^{-3} M treated plants.



b.) Effects of SL on FV/FM ratio of Cu 5.10^{-3} M treated plants.

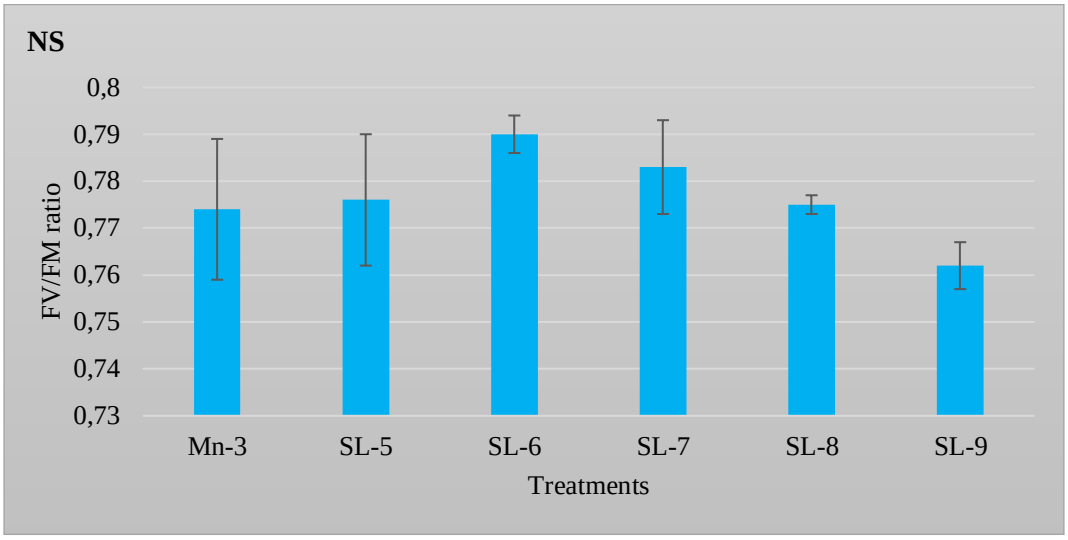


c.) Effects of SL on FV/FM ratio of Cu 10^{-2} M treated plants.

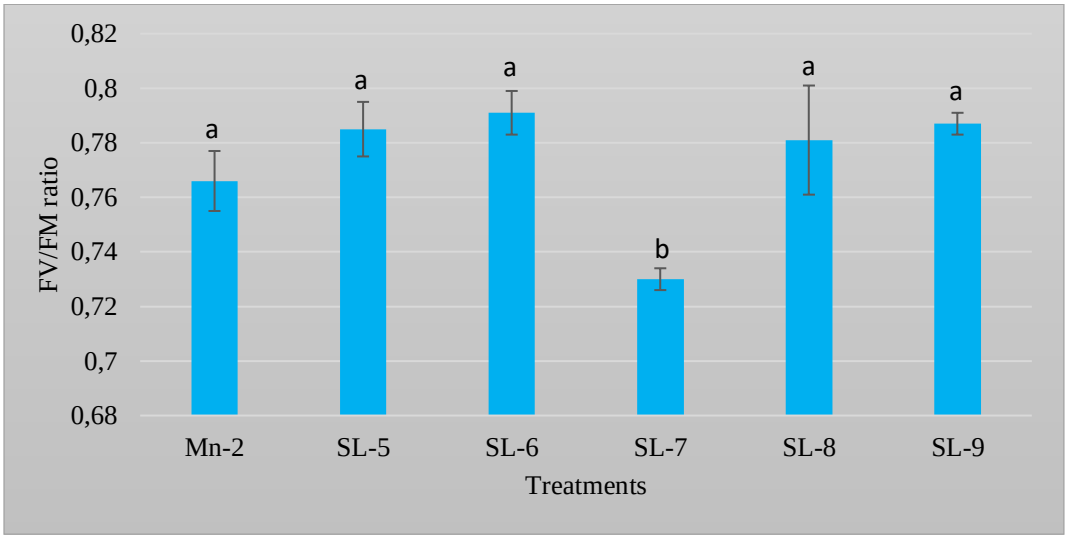
***FIGURE 3.31**: **Possible involvement of SL on the FV/FM ratio of Cu treated plants.** HM treated plants (Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-})^{9} molar concentrations in combination with the various HM applied to the soil, are considered HM/SL treatments. Blue bars indicate the mean value of the FV/FM ratio. Letters above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/SL treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/SL treatments.*

3.2.3f. Possible involvement of SL on the FV/FM ratio of Mn treated plants

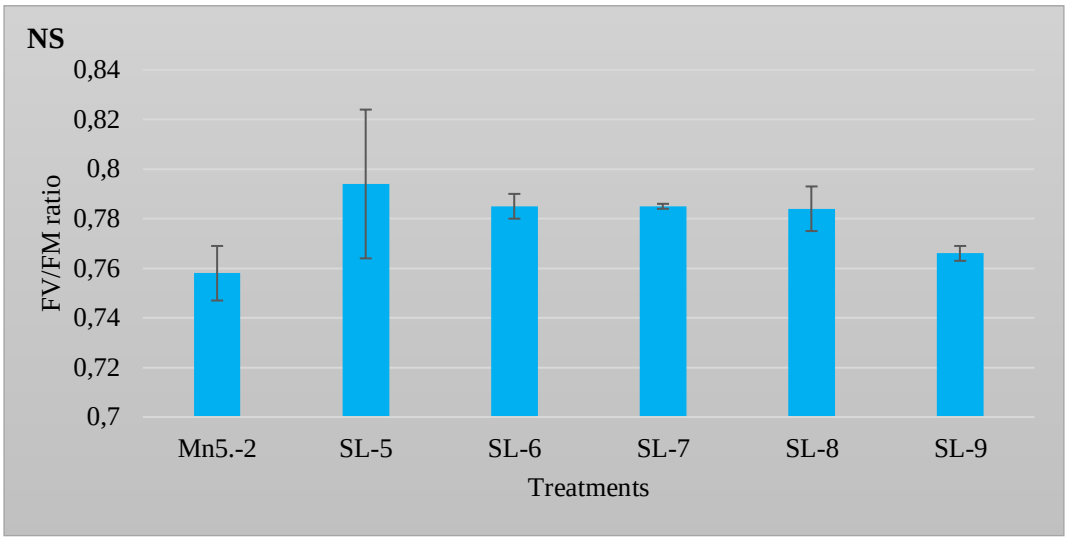
All the concentrations of SL applied on the Mn 10^{-3} M and Mn 5.10^{-2} M treated plants showed no significant influence on the FV/FM ratio of the respective plant shoots (Figure 3.32a and c). SL10^{-7} M significantly decreased the FV/FM ratio of the Mn 10^{-2} M treated plants (Figure 3.32b).



a.) Effects of SL on FV/FM ratio of Mn 10^{-3} M treated plants.



b.) Effects of SL on FV/FM ratio of Mn 10^{-2} M treated plants.

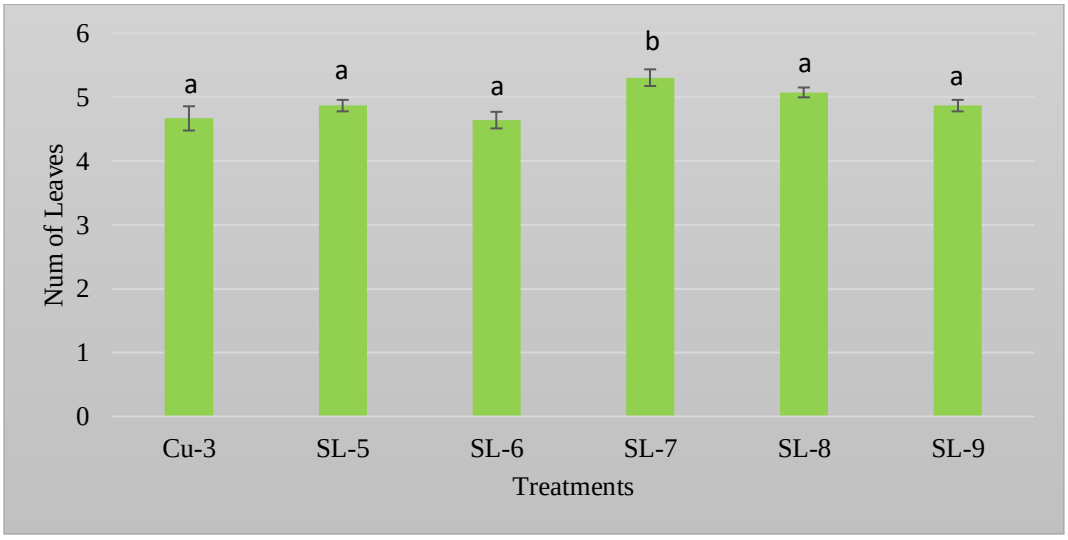


c.) Effects of SL on FV/FM ratio of Mn 5.10^{-2} M treated plants.

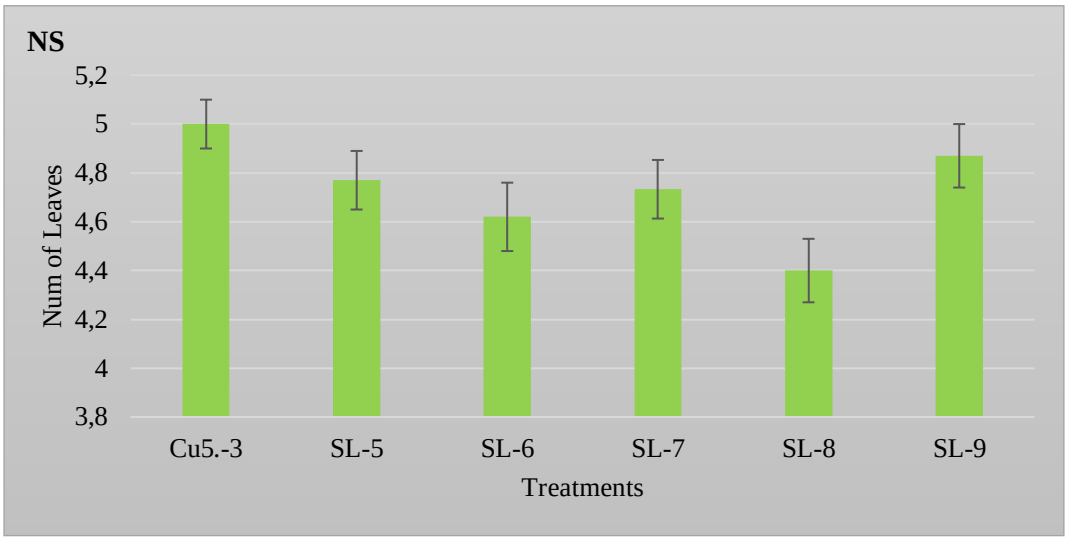
***Figure 3.32**: **Possible involvement of SL on the FV/FM ratio of Mn treated plants**. HM treated plants (Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} molar concentrations in combination with the various HM applied to the soil respectively, are considered HM/SL treatments. Blue bars indicate the mean value of the FV/FM ratio. Letters above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/SL treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/SL treatments.*

3.2.3g. Possible involvement of SL on the number of leaves formed by Cu treated plants

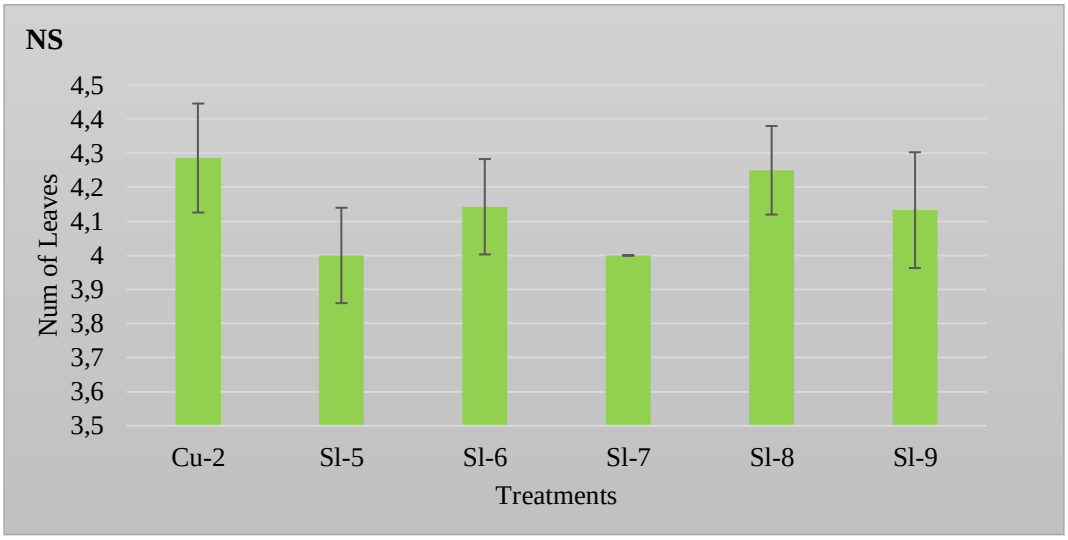
SL 10^{-7} M significantly increased the number of leaves formed by the plants treated with Cu 10^{-3} M (Figure 3.33a). On the other hand, no concentrations of SL applied significantly influenced the number of leaves formed by Cu 5. 10^{-3} M and Cu 10^{-2} M treated plants (Figure 3.33b and c).



a.) Effects of SL on the number of leaves of Cu 10^{-3} M treated plants.



b.) Effects of SL on the number of leaves of Cu 5.10^{-3} M treated plants.

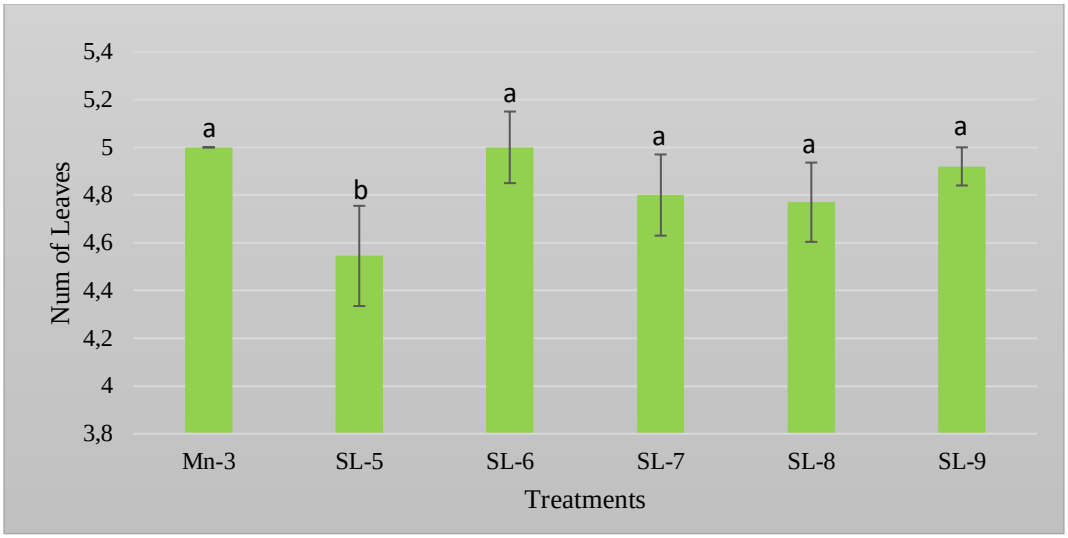


C.) Effects of SL on the number of leaves of Cu 10^{-2} M treated plants

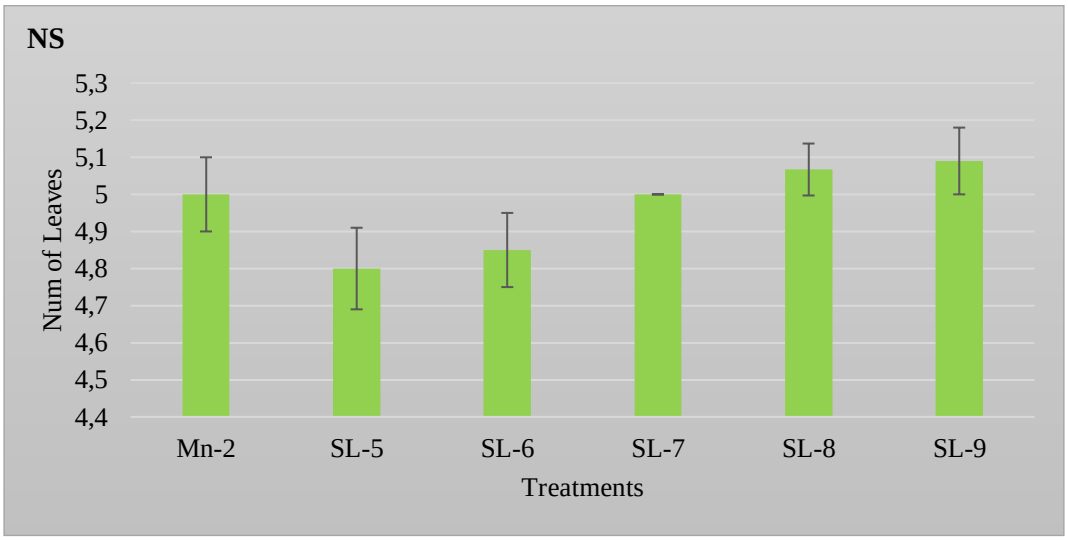
***Figure 3.33*: Possible involvement of SL on the number of leaves formed by Cu treated plants.** *HM treated plants (Cu 10^{-3} (Cu-3), Cu 5.10^{-3} (Cu5.-3) and Cu 10^{-2} (Cu-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations in combination with the various HM in soil, are considered HM/SL treatments. Green bars indicate the mean value of the number of leaves. Letters above the bars indicate significant differences between the mean value of the number of leaves of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/SL treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/SL treatments.*

3.2.3h. Possible involvement of SL on the number of leaves formed by Mn treated plants

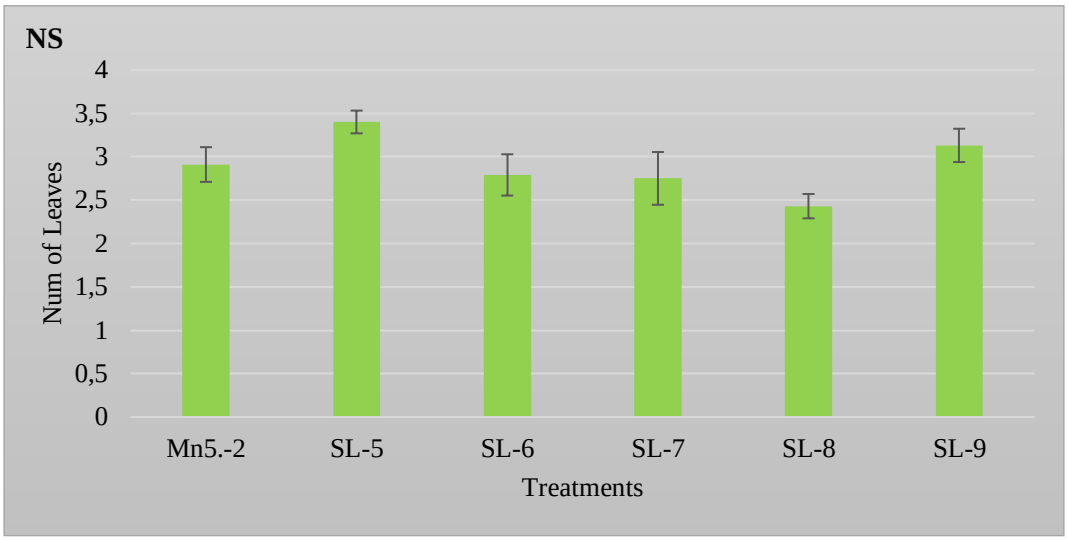
SL 10^{-5} M significantly decreased the number of leaves formed by the plants treated with Mn 10^{-3} M (Figure 3.34a). On the other hand, no concentrations of SL applied significantly influenced the number of leaves formed by Mn 10^{-2} M and Mn 5.10^{-2} M treated plants (Figure 3.34b and c).



a.) Effects of SL on the number of leaves of Mn 10^{-3} M treated plants**.**



b.) Effects of SL on the number of leaves of Mn 10^{-2} M treated plants.



c.) Effects of SL on the number of leaves of Mn 5.10^{-2} M treated plants.

***Figure 3.34*: Possible involvement of SL on the number of leaves formed by Mn treated plants.** *HM treated plants (Mn 10^{-3} (Mn-3), Mn 10^{-2} (Mn-2) and Mn 5.10^{-2} (Mn5.-2)) M, are wheat plants exposed to HM but not treated with SL. Wheat plants treated with SL (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) molar concentrations in combination with the various HM in soil, are considered HM/SL treatments. Green bars indicate the mean value of the number of leaves. Letters above the bars indicate significant differences between the mean value of the number of leaves of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Similar letters over the bars indicate no significant differences between the HM treatment in comparison with HM/SL treatments while different letters indicate significant differences between the HM treatment in comparison with, HM/SL treatments.*

3.3. Results of the cellular tolerance test

To fully understand the level of resilience and plasticity of plants to the environmental pressures in their surroundings, it is crucial to understand the cellular and developmental mechanisms that govern the architecture of plants. Also, a complete understanding of the importance of the roles played by each structure in the physiological functioning of the plants is important (Wei *et al.*, 2016). Plasmolysis was used in this study as a tool to measure the sensitivity and resilience of the cells of the leaves of wheat plants of the various treatments. Treatments considered for the cellular tolerance test in this study included:

1. The highest and lowest concentration of the respective HM treatments ($MnSO_4$ and $CuSO_4$).
2. The highest and lowest concentration of the respective phytohormone treatments JA and SL.
3. Highest and lowest treatment of the respective HM/phytohormone treatments.
4. Control treatments (C).

The results on tables 1 to 4 show the results of the cellular tolerance test of the selected treatments in six different concentrations of HM solutions ($CuSO_4$ and $MnSO_4$) and tap water acting as a C solution. The results displayed on the tables are color-coded in accordance with the degree of plasmolysis showed by the cells. In this study a section is considered to display cellular tolerance when:

a) At least 50-90% of the cells were plasmolyzed (+) and denoted on the tables with green color,
b) partially tolerant (+-) when not less than 10 to 40% of cells were plasmolyzed denoted on the table with a yellow color,
c) and unplasmolyzed (-) < 5 % of cells were plasmolyzed denoted on the table with a red color.

The highest and lowest concentration of the phytohormone treatments was compared to the control treatment while the highest and lowest concentration of the HM/phytohormone treatment was compared to the HM treatment.

3.3.1. Cellular tolerance test for Cu and JA treatments (Table 1).

The cells of the Cu 10^{-2}/ JA 10^{-5} M treatment showed no difference in cellular tolerance when compared to the cells of the Cu 10^{-2} M treatment meanwhile, the cells of the Cu 10^{-2}/ JA 10^{-9} M treatment were more cellular tolerant as compared to the cells of the Cu 10^{-2} M treatment.

The cells of the Cu 10^{-3}/ JA 10^{-5} M treatment showed a significantly low level of cellular tolerance when compared to the cells of the Cu 10^{-3} M treatment. On the other hand, the cells of the Cu 10^{-3}/ JA 10^{-9} M treatment were more cellular tolerant when compared to the cells of the Cu 10^{-3} M treatment.

The cells of the JA 10^{-5} M and 10^{-9} M treatment showed no difference in cellular tolerance when compared to the cells of the C treatment.

The cells of the Cu 10^{-2} M and Cu 10^{-3} M treatment were more tolerant of the HM in solution as compared to the cells of the C treatment.

Table 1: **Cellular tolerance test for Cu and JA treatments.**

Treatments	Cu 10^{-1}	Cu 10^{-2}	Cu 10^{-3}	Cu 10^{-4}	Cu 10^{-5}	Cu 10^{-6}	C (H_2O)
Cu 10^{-2}	-	-	+-	+-	+	+	+
Cu10^{-2}/ JA 10^{-5}	-	-	+-	+-	+	+	+
Cu 10^{-2}/ JA 10^{-9}	-	+-	+-	+	+	+	+
Cu 10^{-3}	-	-	+-	+	+	+	+
Cu 10^{-3}/ JA 10^{-5}	-	-	-	+-	+-	+-	+
Cu 10^{-3}/ JA 10^{-9}	-	+-	+	+	+	+	+
JA 10^{-5}	-	+-	+	+	+	+	+
JA 10^{-9}	-	+-	+	+	+	+	+
C	-	+-	+	+	+	+	+

Table 1: Cellular tolerance test for Cu and JA treatments. *This table shows the results of the cellular tolerance of the selected treatments (Cu 10^{-2}, Cu10^{-2}/ JA 10^{-5}, Cu 10^{-2}/ JA 10^{-9}, Cu 10^{-3}, Cu 10^{-3}/ JA 10^{-5}, Cu 10^{-3}/ JA 10^{-9}, JA 10^{-5}, JA 10^{-9}) M, and C in different solutions of $CuSO_4$ (Cu 10^{-1} to Cu 10^{-6}) M, and water(H_2O) acting as C solution respectively.*

3.3.2. Cellular tolerance test for Mn and JA treatments (Table 2**).**
The cells of the Mn 5.10^{-2}/ JA 10^{-5} M and Mn 5.10^{-2}/ JA 10^{-9} M treatments were more cellular tolerant as compared to the cells of the Mn 5.10^{-2} M treatment respectively.

The cells of the Mn 10^{-3}/ JA 10^{-5} M treatment were significantly less tolerant as compared to the cells of the Mn 10^{-3} M treatment. On the other hand, the cells of the Mn 10^{-3} / JA 10^{-9} M treatment were more cellular tolerant when compared to the cells of the Mn 10^{-3} M treatment.

The cells of the JA 10^{-5} M and 10^{-9} M showed a significant decrease in cellular tolerance when compared to the cells of the C treatment.

The cells of the Mn 5.10^{-2} M and Mn 10^{-3} M treatment were more tolerant of the HM in solution as compared to the cells of the C treatment.

Table 2: **Cellular tolerance test for Mn and JA treatments.**

Treatments	$Mn10^{-1}$	Mn 10^{-2}	Mn 10^{-3}	Mn 10^{-4}	Mn 10^{-5}	Mn 10^{-6}	C (H_2O)
$Mn5.10^{-2}$	-	-	+-	+	+	+	
$Mn5.10^{-2}$/ JA 10^{-5}	-	+-	+	+	+	+	+
$Mn5.10^{-2}$/ JA 10^{-9}	-	+-	+	+	+	+	+
Mn 10^{-3}	-	+-	+	+	+	+	+
$Mn10^{-3}$/ JA 10^{-5}	-	-	+-	+-	+-	+	+
$Mn10^{-3}$ / JA 10^{-9}	-	+	+	+	+	+	+
JA 10^{-5}	-	-	-	-	-	+-	+
JA 10^{-9}	-	-	-	-	+-	+-	+
C	-	-	-	+	+	+	+

*Table 2: **Cellular tolerance test for Mn and JA treatments.** This table shows the results of the cellular tolerance of the selected treatments (Mn 10^{-2}, Mn 10^{-2}/ JA 10^{-5}, Mn 10^{-2}/ JA 10^{-9}, Mn 10^{-3}, Mn 10^{-3}/ JA 10^{-5}, Mn 10^{-3}/ JA 10^{-9}, JA 10^{-5}, JA 10^{-9}) M and C in different solutions of $MnSO_4$ (Mn 10^{-1} to Mn 10^{-6}) M, and water(H_2O) acting as C solution respectively.*

3.3.3. Cellular tolerance test for Cu and SL treatments (Table 3).

The cells of the $Cu10^{-2}$/ SL 10^{-5} M treatment showed no difference in cellular tolerance when compared to the cells of the Cu 10^{-2} M treatment, while the cells of the $Cu10^{-2}$/ SL 10^{-9} M treatment were significantly more cellular tolerant as compared to the cells of the Cu 10^{-2} M treatment.

The cells of the Cu 10^{-3}/ SL 10^{-5} M treatment were less cellular tolerant as compared to the cells of the Cu 10^{-3} M treatment meanwhile, the cells of the Cu 10^{-3}/ SL 10^{-9} M treatment were significantly more cellular tolerant when compared to the cells of the Cu 10^{-3} M treatment.

The cells of the SL 10^{-5} M treatment showed a higher cellular tolerance as compared to the cells of the C treatment. On the other hand, the cells of the SL 10^{-9} M treatment were significantly more tolerant than the cells of the C treatment.

The cells of the Cu 10^{-2} M and Cu 10^{-3} M treatment were more tolerant of the HM in solution as compared to the cells of the C treatment.

Table 3: **Cellular tolerance test for Cu and SL treatments.**

Treatments	Cu 10^{-1}	Cu 10^{-2}	Cu 10^{-3}	Cu 10^{-4}	Cu 10^{-5}	Cu 10^{-6}	C (H_2O)
Cu 10^{-2}	-	-	-	+	+	+	+
Cu 10^{-2}/SL10^{-5}	-	-	-	+	+	+	+
Cu 10^{-2}/SL10^{-9}	-	+	+	+	+	+	+
Cu 10^{-3}	-	-	+-	+	+	+	+
Cu 10^{-3}/SL10^{-5}	-	-	+-	+-	+	+	+
Cu 10^{-3}/SL10^{-9}	-	+	+	+	+	+	+
SL 10^{-5}	-	-	+-	+-	+	+	+
SL10^{-9}	-	-	+	+	+	+	+
C	-	-	-	-	+-	+	+

*Table 3: **Cellular tolerance test for Cu and SL treatments**. This table shows the results of the cellular tolerance of the selected treatments (Cu 10^{-2}, $Cu10^{-2}$/ SL 10^{-5}, Cu 10^{-2}/ SL 10^{-9}, Cu 10^{-3}, Cu 10^{-3}/ SL 10^{-5}, Cu 10^{-3}/ SL 10^{-9}, SL 10^{-5}, SL 10^{-9}) M and C in different solutions of $CuSO_4$ (Cu 10^{-1} to Cu 10^{-2}) M, and water(H_2O) acting as C solution respectively.*

3.3.4. Cellular tolerance test for Mn and SL treatments (Table 4).

The cells of the Mn 5.10^{-2}/ SL 10^{-5} M treatment were less cellular tolerant as compared to the cells of the Mn 5.10^{-2} M treatment respectively. On the other hand, the cells of the Mn 5.10^{-2}/ SL 10^{-9} M treatment were slightly more tolerant as compared to the cells of the Mn 5.10^{-2} M treatment.

The cells of the Mn 10^{-3}/ JA 10^{-5} M treatment showed less cellular tolerance as compared to the cells of the Mn 10^{-3} M treatment. On the other hand, the cells of the Mn 10^{-3} / JA 10^{-9} M treatment were significantly more tolerant than the cells of the Mn 10^{-3} M treatment.

The cells of the SL 10^{-5} M treatment showed a decreased level of cellular tolerance as compared to the cells of the C treatment. On the other hand, the cells of the SL 10^{-9} M treatment were more tolerant than the cells of the C treatment.

The cells of the Mn 5.10^{-2} M and Mn 10^{-3} M treatment were more tolerant of the HM in solution as compared to the cells of the C treatment.

Table 4: **Cellular tolerance test for Mn and SL treatments.**

Treatments	$Mn10^{-1}$	$Mn10^{-2}$	$Mn10^{-3}$	$Mn10^{-4}$	$Mn10^{-5}$	$Mn10^{-6}$	C (H_2O)
$Mn5.10^{-2}$	-	-	+-	+	+	+	+
$Mn5.10^{-2}/SL10^{-5}$	-	-	+-	+-	+	+	+
$Mn5.10^{-2}/SL10^{-9}$	-	+-	+-	+	+	+	+
Mn 10^{-3}	-	-	+	+	+	+	+
$Mn10^{-}3/SL10^{-5}$	-	-	+-	+	+	+	+
$Mn10^{-3}$ /$SL10^{-9}$	-	+	+	+	+	+	+
SL 10^{-5}	-	-	-	+-	+	+	+
SL 10^{-9}	-	-	+-	+	+	+	+
C	-	-	-	+-	+	+	+

*Table 4: **Cellular tolerance test for Mn and SL treatments.** This table shows the results of the cellular tolerance of the selected treatments (Mn 10^{-2}, Mn 10^{-2}/ SL 10^{-5}, Mn 10^{-2}/ SL 10^{-9}, Mn 10^{-3}, Mn 10^{-3}/ SL 10^{-5}, Mn 10^{-3}/ SL 10^{-9}, SL 10^{-5}, SL 10^{-9}) M, and C in different solutions of $MnSO_4$ (Mn 10^{-1} to Mn 10^{-2}) M, and water(H_2O) acting as C solution respectively.*

4. Discussion

The aim of this study was to examine the effects of different concentrations of Cu and Mn on wheat and to further investigate (a) the possible involvement of phytohormones JA and SL on the growth improvement and (b) stress tolerance of wheat plants to these respective HMs. To do this wheat plants were grown in soil containing Cu and Mn and to these plants, the respective phytohormones were applied. The experiment was structured into treatment groups which included, the control (C), the HM treatment, the phytohormone treatment and the HM/phytohormone treatments. For statistical analysis treatment groups were replicated three times. Plant parameters investigated were root and shoot length, root and shoot weight, FV/FM ratio, number of leaves and cellular tolerance. To answer questions pertaining to the effects of HM on the plants, the parameters of the HM treatments were compared to the parameters of the C treatment. To answer questions pertaining to the involvement of phytohormones on the phytohormone treatments, the parameters of the phytohormone treatments were compared to the parameters of the C treatment, and most importantly, to answer questions pertaining to the involvement of phytohormones on the stress tolerance of the respective HM, the HM/phytohormone treatments were compared to the HM treatments.

4.1. Effects of Cu on wheat

Within the confines of a plant cell, Cu is required in at least six locations, for example, the cytosol and the endoplasmic reticulum. The concentration of Cu is kept at a very strict threshold value above which toxicity occurs immediately (e.g. Yruela 2005). In the nutrient media Cu concentrations usually ranges from 10^{-14} to 10^{-16} M above and below which there is an excess or deficiency. In the soil Cu concentrations usually range from 10^{-6} to 10^{-9} M (e.g. Marschner 1995). In plant cells, the concentration of Cu is maintained at a strict equilibrium above which free Cu in the cell can hamper growth and come in the way of important cellular processes such as photosynthesis and respiration (e.g. Marschner 1995; Prasad and Strzalka 1999; Yruela 2005). Plants exposed to high concentrations of Cu (3-100 mM) are usually observed to show very common observation characteristics such as chlorotic symptoms, necrotic symptoms, and inhibition of shoot and root growth (Quartacci, Cosi and Navari-Izzo, 2001). These are very important parameters to study when considering the effects of HMs on plants. In this study, the effects of Cu concentrations of 10^{-3} M, 5.10^{-3} M and 10^{-2} M on wheat were investigated. Plants grown on soil treated with Cu 10^{-3} M showed with respect to the C treatment an insignificant decrease in the shoot and root length, shoot weight and number of leaves. There was a significant decrease in the root weight of Cu 10^{-3} M treated plants in comparison to the C. In addition, there was an insignificant increase in the FV/FM ratio in comparison with the FV/FM ratio of the C treatment. These insignificant differences showed indicated that wheat plants were able to stimulate stress defense mechanisms within their system to cope with Cu concentrations of 10^{-3} M, given that the typical Cu concentrations in the soil range from 10^{-6} to 10^{-9} M (e.g. Marschner 1995) and the concentration 10^{-3} M, in this case, lies above that. It is postulated here that, a possible explanation as to why the plants exhibited tolerance at this Cu concentration in the soil could be that; the plants might have developed the ability to restrict the movement of the Cu ions up to its aerial parts. This could have been made possible by the plant maintaining low and constant metal concentrations over a broad range of metal concentrations in soil, principally by holding metals in their root system (Cunningham, 1995). Additionally, the production of reactive oxygen species (ROS) due to Cu stress, could be one of the reasons for the acclimation of the wheat plants to the Cu 10^{-3} M stress-induced. It is important to note here that increased production of ROS is also a toxic byproduct of stress metabolism which can only be detoxified by glutathione (GSH). The detoxification of ROS is GSH dependent. This reductive activity eliminates ROS produced either directly or indirectly by metal toxicity and this mechanism must be kept at equilibrium in the cells, to avoid cellular injure (Hasanuzzaman *et al.*, 2017). Wheat plants exposed to Cu 10^{-3} M showed insignificant differences in the different parameters measured which may have been due to the fact that ROS produced was enough to induce acclimation in the HM induced cells and only moderate imbalances of the GSH/ROS detox mechanism might have been brought about by this metal concentration. On the other hand, there was a significant decrease in the root weight of Cu 10^{-3} M treatment. We suggest here that given that the roots are always the first to get into contact with HMs in the soil and thus their cells are directly affected which could either be by losing elasticity and thus unable to expand or failure to produce root hairs and lateral roots, this might have contributed to the significant decrease in root weight showed at this concentration. This observation has previously been reported in wheat where in Cu lead to a significant decrease in the root weight (Quartacci, Cosi and Navari-Izzo, 2001). Furthermore, there was an insignificant increase in the FV/FM ratio in comparison with the C

treatment. It can be proposed that at this concentration of the HM treatment, the photosynthetic apparatus which normally appears to be very sensitive to the toxicity of heavy metals, was not negatively affected by Cu 10^{-3} M. Generally high concentrations of HMs invariably affect the photosynthetic functions either by directly obstructing the enzyme activities of the Calvin cycle or indirectly by causing CO_2 deficiency due to stomatal closure (e.g. Linger, Ostwald and Haensler, 2005). This observation might have been due to the plant's ability to prevent the uptake of the metals to the aerial parts (Baker, Reeves and Hajar, 1994; Raskin *et al.*, 1994) or in the case where some pass through in excess to the leaf cells, store them in specialized compartments (Schneider *et al.*, 2009) to avoid disorganization of the chloroplast ultrastructure and inhibition of electron transport processes.
The Cu 5.10^{-3} M treated plants showed with respect to the C treatment a significant decrease in the root and shoot length, and, root and shoot weight. On the other hand, there was an insignificant increase in the FV/FM ratio, and the number of leaves in comparison with the C treatment. Again, it is important to note that typical Cu concentrations in the soil range from 10^{-6} to 10^{-9} M (e.g. Marschner 1995) and the concentration of Cu 5.10^{-3} M lies above that. We, therefore, suggest here that the significant decrease in plant biomass could be due to the inability of the plants to allocate enough energy for growth given that most of its energy was spent on exhibiting tolerance to the stress-induced. Generally, plant biomass is an indicator of crop productivity in terms of dry matter yield. The increased photosynthetic process is considered as the basis for the building up of organic substances which accounts for 80–90% of the total dry matter of plants (Bishnoi *et al.*, 1993; Bishnoi, Chugh and Sawhney, 1993). However, heavy metals have been well reported to reduce biomass production (Tokalioğlu and Kartal, 2006), mainly due to their interference with the photosynthetic activity. Interestingly, on the other hand, there was an insignificant decrease in the FV/FM ratio and the number of leaves counted which was not expected given the significant decrease in the plant biomass. We propose that even though there is an increase in the fluorescence of the chloroplast other parameters that are implicated in the photosynthetic activity such as water and nutrient uptake from the roots were limited due to the effects of the Cu on the roots and thus an inefficiency in the photosynthetic ability to increase biomass buildup.

The Cu 10^{-2} M treated plants showed in relation to the C treatment a significant decrease in all parameters measured. Here it is very trivial to mention that typical Cu concentration in the soil range from 10^{-6} to 10^{-9} M (e.g. Marschner 1995) and the concentration 10^{-2} M lies way above this threshold value. There is no denying that Cu is essential for plants and can even be tolerated at increased concentrations in soil solution. For example, in this study, the Cu 10^{-3} M treatment which was above the threshold value of normal soil solution concentrations, did not adversely cause stress effects on the plants. Nevertheless, increased levels of Cu in the soil cannot be tolerated by plants in general and usually show toxic effects. In this study, the treatment with Cu 10^{-2} M caused a significant decrease in all the respective parameters measured. Reasons for this could be increased levels of Cu in the cell thus cellular toxicity, increased ROS build up in the cell thus distorting the equilibrium of the ROS/GSH detox mechanism causing lipid peroxidation, negative effects of Cu on the photosynthetic apparatus thus invariably affecting the photosynthetic functions of the plants either directly or indirectly by inhibiting the enzyme activities of the Calvin cycle, and CO_2 deficiency due to stomatal closure (e.g. Linger, Ostwald and Haensler, 2005), interference with the proper functioning of micronutrients (Zayed and Terry, 2003a), negative effects of Cu on the root hydraulic conductivity (Poschenrieder, Gunsé and Barceló, 1989), etc. All of these reasons and more could be the possible reason for the general decrease in all parameters measured at this treatment concentration.

4.2. Effects of Mn on wheat

As an essential micronutrient, Mn participates in the structure of photosynthetic proteins and enzymes. Therefore, low levels of Mn are absolutely necessary for normal nutrition and development of plants. Principally because, it delivers the essential electrons for photosynthesis (Buchanan, Gruissem and Jones, 2000) Nonetheless important processes such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe and P), in the plant tissue, can be altered by the presence of Mn concentrations above an accepted range causing oxidative stress (Ducic and Polle, 2005; Lei, Korpelainen and Li, 2007). The upper limit of Mn injury, as well as the level of tolerance exhibited by a plant to an excess of this metal, is to a great extend dependent on the plant species and cultivars or genotypes within a species (e.g. Foy, Chaney and White, 1978). In this study, the effects of Mn concentrations of 10^{-3} M, 10^{-2} M and 5.10^{-2} M on wheat plants were investigated.
Plants grown on soil treated with Mn 10^{-3} M showed in relation to the C treatment an insignificant increase in all parameters measured. given that the concentration of Mn in soil solutions varies widely

from 10^{-9} M to $l0^{-3}$ M with most soils in the range of 10^{-7} to $l0^{-5}$ M. Mn 10^{-3} M falls within the range of some soils and slightly above the threshold value of most soils. Therefore, we can suggest here that a possible explanation for the insignificant increases in plant parameters by Mn 10^{-3} M treatment here had to do with the HM at this concentration not inducing stress but acting as a growth promoter, improving all growth parameters measured. Additionally, Mn at this concentration may possibly have been acting as an essential micronutrient participating in the structure of photosynthetic proteins and enzymes thus normal nutrition and development of plants or in the chloroplasts affecting the water-splitting system of photosystem II (PSII), thereby providing the necessary electrons for photosynthesis (Buchanan, Gruissem and Jones, 2000) thus improving growth parameters of the plants.

The Mn 10^{-2} M treated plants showed with respect to the C treatment variable increases and decreases in the root and shoot length and weight. The FV/FM ratio and the number of leaves also showed an insignificant decrease in comparison to the C treatment. It is important to have in mind that the treatment of the plants with Mn 10^{-2} M lies above the normal threshold concentration of Mn in soil solutions which normally varies widely from 10^{-9} to $l0^{-3}$ M, with most soils in the range of 10^{-7} M to $l0^{-5}$ M. Therefore, it was expected that the growth parameters studied decrease as a result of the plants allocating more energy on stress tolerance than growth. We suggest here that the insignificant increases and decreases of the various parameters showed demonstrated the plant's ability to withstand the stress-induced on its metabolism by the HMs. On the other hand, we also postulate that the significant decreases showed for some plant parameters measured were due to the plant's lack of ability to sustain growth in the presence of the HM treatment and at the same time survive the stress inflicted on its system. Treatments with Mn 10^{-2} M generally imparted significant effects on the root system of the plants. This was expected given that roots are the first to get in contact with HM in the soil forming a soil/root barrier protecting the delicate shoot system (Cunningham, 1995).

Plants grown in soil treated with Mn 5.10^{-2} M showed in relation to the C a significant decrease of all parameters measured. Given that this treatment concentration is way above the threshold value of Mn in soil required for plant absorption, significant stress levels were imparted on the plant metabolism. Possible reasons for this observation will definitely be linked to the concentration of the Mn treatment which might have caused variations in the photosynthetic ability of the plants, changing various processes, such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe, and P), bringing about oxidative stress (Ducic and Polle, 2005; Lei, Korpelainen and Li, 2007). Additionally, increased levels of Mn in the cell thus increased ROS build up distorting the equilibrium of the ROS/GSH detox mechanism leading to lipid peroxidation. Additionally, toxic levels of Mn caused negative effects on the photosynthetic ability of the plants either directly by inhibiting the enzyme activities of the Calvin cycle or indirectly by causing a CO_2 deficiency in the cell due to stomatal closure (e.g. Linger, Ostwald and Haensler, 2005). Interference with the proper functioning of micronutrients (Zayed and Terry, 2003a), negative effects of Mn on the root hydraulic conductivity (e.g. Poschenrieder, Gunsé and Barceló, 1989), etc. All of these reasons and more could be the possible reasons for the general decrease in all parameters measured at this treatment concentration.

4.3. involvement of phytohormones in HM induced stress

Phytohormones are essential chemical ingredients that integrate endogenous developmental cues with environmental signals to regulate plant growth, development, and defense. They are effective at very low concentrations which could be directly in the plant's part where produced or indirectly in other plant parts where they are transferred to (Öktüren and Sönmez, 2005). In this study phytohormones, JA and SL were applied on wheat plants to investigate; (1) the effects of the respective phytohormones on wheat plants grown in optimal environmental conditions and (2) the involvement of the respective phytohormones on the stress tolerance of wheat to Mn and Cu. The main objective of the foliar application of phytohormones was to; (a) improve plant growth parameters under HM induced stress, (b) stimulate plant metabolism, (c) protect the plants from abiotic stress, (d) enhance soil microbial activity, and (d) optimize nutrient uptake assimilation and efficiency (Figure 4.1).

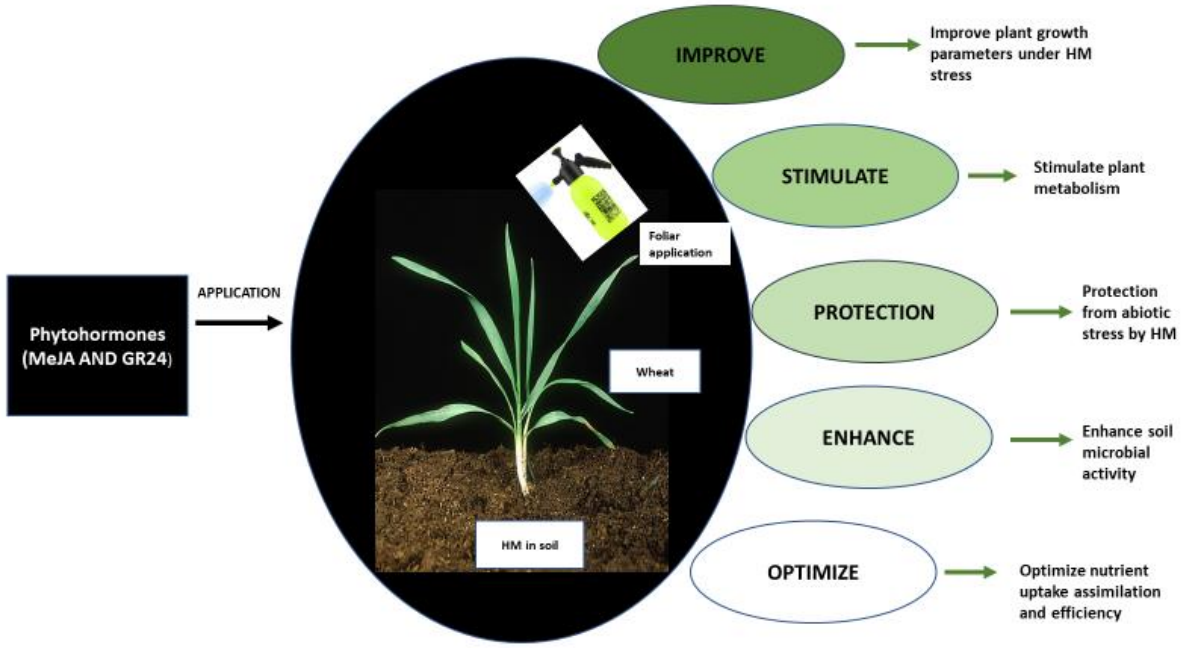


Figure 4.1. Expectations of exogenous application of phytohormones. *Foliar application of phytohormones brings about crosstalk's with other phytohormones in the plant leading to improvement, stimulation, protection, enhancement, and optimization of different plant parameters enabling them to cope with different types of biotic and abiotic stresses.*

Firstly, to respond to questions pertaining to the possible involvement of exogenously applied phytohormones on the growth performance of wheat plants growing under optimal environmental conditions, the respective phytohormone treatments were compared to the C treatment. Secondly, to respond to questions pertaining to the possible involvement of phytohormones on the stress tolerance of wheat plants to HM, the HM treatments were compared to the HM/phytohormone treatments. In this study, generally, the exogenous application of the different concentrations of the respective phytohormones on wheat plants showed varying effects on the plant parameters measured in an inconsistent manner.

4.3.1. Possible involvement of JA on wheat

the core of plant stress responses is centered around the interactions between phytohormones and other phytohormones (He *et al.*, 2017). JA does not work independently but acts in a complex signaling network combined with other plant hormone signaling pathways (e.g. Wasternack and Strnad, 2016). Previous studies have well documented that upon activation of JA biosynthesis and signaling in plants, plants defense mechanisms such as the promoting of trichome formation following wounding or insect attack in cross-talk with Gibberellic Acid (GA), regulation of stomatal closure and reopening to regulate water loss, gas exchange, and plant immunity to pathogens are key roles of JA acting in plants enabling them to combat abiotic and biotic stress. Some examples of previous studies which have reported the involvement of JA to eliminate stress include; elimination of salt stress by jasmonates thereby

recovering salt inhibition on dry mass production in rice (Kang *et al.*, 2005), JA delayed the ABA-mediated inhibition of seed germination in Arabidopsis (Ellis and Turner, 2002), inhibition of apical hook formation (Song *et al.*, 2014), delay of flowering (Zhai *et al.*, 2015). inhibition of petal expansion (Brioudes *et al.*, 2009; Reeves *et al.*, 2012), induction of leaf senescence (Qi *et al.*, 2015), etc. Generally, the inhibitory effect of JA on growth parameters enhances survival in natural environments by allowing plants to concentrate on defending themselves against various stresses.

In this first experiment, two hypotheses were tested for the effects of JA.

1. The effects of JA on phytohormone treatments in comparison to the C treatment and
2. The effects of JA on HM/phytohormone treatments in comparison to HM treated plants.

4.3.1a. Possible involvement of JA on phytohormone treatments

JA 10^{-5} M and 10^{-6} M, treatments significantly decreased the root length while 10^{-6} M, 10^{-7} M and 10^{-8} M, significantly increased the shoot length in comparison to the C treatment. JA 10^{-5} M significantly decreased the root dry weight while no significant difference was shown on the dry weight of the shoots in comparison to the C treatment. There was no significant influence of JA on the FV/FM ratio and the number of leaves.

We propose here that the significant decrease of the root parameters could possibly be due to a significant decrease in the root endophytic community. Previous studies have revealed that upon exogenous application of JA, for example in *Arabidopsis thaliana* (Staswick, Su and Howell, 1992; Yan *et al.*, 2016) and sunflower (*Helianthus annuuss L.*) (Lenzi *et al.*, 1995), has resulted into inhibition of different root parameters. The insignificant differences showed by the FV/FM ratio and the number of leaves could be due to the fact that plants were in optimal growth conditions as such JAs signaling and response did not influence the photosynthetic activity. Previous studies have reported that under increased stress levels exogenous application of JA increased photosynthesis rate e.g. JA application enhanced chlorophyll content by neutralizing the inhibitory effect of salt stress on pigmentation, this was mainly by JA causing a reduction in free proline content which was increased by NaCl application (Yoon *et al.*, 2009).

4.3.1b. Possible involvement of JA on Cu induced stress in wheat

In this study, it was well established that wheat plants exposed to Cu at different concentrations showed varying levels of stress on the different plant parameters measured.

For the plants treated with Cu 10^{-3} M, JA showed no significant influence on the plant's root/shoot length, and weight, and the number of leaves. On the other hand, there was a substantial increase in the FV/FM ratio in comparison with the FV/FM ratio of the Cu treatment. This significant influence of JA on the FV/FM ratio could be linked to the action of JA enhancing the chlorophyll content by neutralizing the inhibitory effect of HM stress on pigmentation which could mainly be brought about by JA causing a reduction in free proline content which is generally increased in plants when exposed to stress in our case HM. This was demonstrated in soybean with NaCl (Yoon *et al.*, 2009).

For the plants exposed to Cu 5.10^{-3} M in the soil, JA 10^{-6} M induced a significant decrease in the root length. On the other hand, the concentrations 10^{-8} M and 10^{-9} M respectively significantly increased the root length in comparison to the HM treated plants. There was no significant difference in the shoot length, shoot and root weight. There was a significant decrease in the FV/FM ratio for the treatments of JA 10^{-6} M and a significant increase in the number of leaves with the JA 10^{-9} M treatments respectively. A possible reason for the significant influence of JA on the roots could be the cross-talks between ABA and JA signaling pathways which have been reported to participate in plant's responses to stress. Specifically here the MYC TFs (JAZs-MYC2) participates in the crosstalk between JA and ABA signaling pathways, affecting plant root growth and defense (Chen *et al.*, 2011). Furthermore, the significant influences of JA showed by the FV/FM ratio of the Cu treatment could be due to the influences JA has on the chlorophyll content (Yoon *et al.*, 2009).

For the plants exposed to Cu 10^{-2} M in the soil, there was no significant influence of JA application on the root length and weight and the FV/FM ratio. On the other hand, JA treatments 10^{-6} M and 10^{-7} M significantly decreased the shoot length as well as the shoot weight in comparison to the Cu treatment. This significant influence of JA on the shoot length and weight can be explained by the fact that due to the significant effects of stress brought on the plants by exposure to Cu 10^{-2} M, which is inherently a very high concentration, JA acted in this case as a repressor of shoot growth thus enabling the plant to focus more energy on defending itself against the HM induced stress Previous studies have well established that, MYC TFs (MYC2, MYC3, and MYC4) adversely regulate gene expression in the cell cycle, in contrast to the constructive regulation of JAZ in plant growth, thereby impeding plant growth

(e.g. Gasperini *et al.*, 2015). The general switch of the JA signaling pathway causes MYC2 which is famously known as the main JA TF to participate in cross-talks with JA, ABA, auxin, ET, GA, and other signaling pathways (Huang *et al.*, 2017). This interaction represses plant growth and enables plants to cope with abiotic and biotic stress. This is in line with previous studies on Arabidopsis, whereby the inhibitory effect of JA on growth enhanced survival in natural environments under stress by allowing plants to concentrate on defending themselves against various stresses (Hou *et al.*, 2010).

4.3.1c. Possible involvement of JA on Mn induced stress in wheat

Plant`s root and shoot lengths treated with Mn 10^{-3} M were significantly increased by the application of JA concentrations of 10^{-5} M and 10^{-7} M respectively. No significant influence of JA was showed by the root and shoot weight, FV/FM ratio and the number of leaves. Given that Mn 10^{-3} M showed more of a growth stimulatory effect on the plants, it is safe to state that the insignificant influence of JA here on the different plant parameters studied could be due to the fact that JA signaling and transduction was not significantly influenced due to the obvious absence of stress on the plants. On the other hand, significant influences of JA on the shoot and root length could be due to cross-talks between JA and BR (Choudhary *et al.*, 2012). In optimal growth conditions, JA and BR act in a crosstalk's relationship significantly influencing shoot and root architecture.

JA significantly influenced the root length and FV/FM ratio of Mn 10^{-2} M treated plants with respect to the HM/Mn treated plants. On the other hand, the root length, shoot, and root weight, as well as the number of leaves were insignificantly influenced by the JA application. This significant influence of JA 10^{-9} M on the root length and not root weight could be due to the influence of JA on the cell size and not cell number (Biondi *et al.*, 2001). Furthermore, this significant influence on the various plant parameters at this concentration of Mn in soil could be due to cross-talks with other hormones such as GA and abscisic acid. Furthermore, the significant influence of JA on FV/FM ratio could be due to the influence JA has on the chlorophyll content (Yoon *et al.*, 2009)

For the plants treated with Mn 5.10^{-2} M, all concentrations of JA applied showed significant influences on the root length. There was significant involvement in the shoot length with treatments of JA 10^{-7} and 10^{-9}. On the other hand, there was no significant involvement of JA on the FV/FM ratio and the number of leaves. This significant increment in root and shoot length of these plants exposed to high levels of stress might be the activity of JA controlling secondary root growth in crosstalk with auxin to increase mineral uptake from the soil (Creelman and Mullet, 1995; Yoon *et al.*, 2009). Additionally, it has been well reported that under high levels of stress JA acts in crosstalk's with other phytohormones mainly on the root system JA and GA Phytohormone signaling pathways either coordinatively or antagonistically control the growth of plant and their defense responses; however plant defense comes about as a tradeoff to plant growth (e.g. Yang *et al.*, 2012). Also, crosstalk between JA and Auxin has been reported to influence root growth in plants exposed to stress. The crosstalk between JA and ABA signaling pathways involves the participation of JAZs-MYC2 which affects growth and defense (Chen *et al.*, 2011).

4.3.2. Possible involvement of SL on wheat

Strigolactones (SLs) are isolated from the root exudates of plants. They are considered to belong to a group of lactones called carotenoid-derived terpenoid lactones. These root exudates have been proven to have the ability to stimulate seed germination of root parasitic plants such as Striga (Cook *et al.*, 1972; Auger *et al.*, 2012). They have been suggested to play a pivotal role in the regulation of above-ground plant architecture and root development (Koltai *et al.*, 2010; Brewer, Koltai and Beveridge, 2013). One of the most important characteristics of SL which in our times is now being reported to have great applicability in agriculture and stress tolerance is the ability of SL to shape the root architecture by inducing symbiosis with endomycorrhizal fungi (Liu. Guowei *et al.*, 2017). Exogenous application of SL, especially GR24, a synthesized Strigolactone. in most cases has been well studied to be involved in stress responses in plants. For example, SL can augment the drought and salt tolerance of *Arabidopsis* (Ha *et al.*, 2014; Kapulnik and Koltai, 2014). We suppose here that given that drought and salt stress impact similar abiotic stresses on plants as heavy metals SL will alleviate stresses induced on wheat plants by HM.
In this second experiment, two hypotheses were tested for the effects of SL.

1. The effects of SL on phytohormone treatments in comparison to the C treatments and,
2. The effects of SL on HM/phytohormone treatments in comparison to HM treatment.

4.3.2a. Possible involvement of SL on phytohormone treatments

A significant increase in shoot weight was shown by the plants growing under optimal environmental conditions with treatments of SL 10^{-8} M. On the other hand, a significant decrease in root weight was showed by these groups of plants with treatment 10^{-6} M of SL in comparison to C treatment respectively. No significant influence of SL was showed by the root and shoot length, FV/FM ratio and the number of leaves respectively. This significant influence of SL on the dry weight of the roots and shoots could possibly be brought about by the fact that SL signaling in plants plays a pivotal role in the architecture of the roots and shoots which has thus far been well reported in other studies like Crawford *et al.,* (2010) and Domagalska and Leyser (2011).

4.3.2b. Possible involvement of SL on Cu induced stress in wheat

In this experiment, it was well established that wheat plants exposed to different concentrations of Cu showed varying levels of stress on the different plant parameters measured. Different concentrations of SL influenced the different parameters studied differently.

SL significantly decreased the shoot length of Cu 10^{-3} M treated plants at the concentration of 10^{-5} M SL treatment. Also, the number of leaves was significantly increased with treatments of 10^{-7} M SL. On the other hand, there was no significant impact of SL on the root length and weight as well as shoot weight. For the FV/FM ratio, SL showed no significant influence as well. Here we suggest that the significant influence of SL on shoot length showed here might be due to the ability of SL working in crosstalk with other hormones such as auxins to influence the shoot architecture. It is worth noting that even though it has been well established that Strigolactones act as growth inhibitors, this is not always the case for example, surprisingly, inter-fascicular cambium development has been reported to be promoted by SL application (Agusti *et al.,* 2012). On the other hand, the significant influences on the number of leaves could be due to the crosstalk activities between cytokinin and SL which act antagonistically influencing the shoot architecture as well (Dun *et al.,* 2012).

SL concentrations of 10^{-5} M and 10^{-6} M significantly decreased the root weight of Cu 5.10^{-3} M treated plants. The FV/FM ratio was also significantly decreased by the application of SL 10^{-5} M concentration. On the other hand, the root weight, length, and shoot weight were not influenced by SL application. The significant influence of SL on the roots could be a result of the plant's response to the stress induced by initiating cross talks with other phytohormones such as ethylene, auxin, and cytokinin (Ha *et al.,* 2014; Kapulnik and Koltai, 2014). Also, SL plays a major role in the architecture of the root under suboptimal environmental conditions; for example, under low-phosphate growth conditions, it has been shown that elevated levels of SL in plants repressed shoot branching (Umehara *et al.*, 2010; Kohlen *et al.*, 2011), increased lateral root formation (Ruyter-Spira *et al.*, 2011), and promoted root hair density (Mayzlish-Gati *et al.*, 2012). Not surprisingly, mutants defective in the SL pathway are less able to respond to low phosphate (Umehara *et al.*, 2008; Kohlen *et al.*, 2011). On the other hand, the significant influence of SL on the FV/FM ratio can be purported to be due to the ability of SL to influence hormonal changes in the plant that affect the chloroplast which is the site for most of the photosynthetic activities (Ma *et al.*, 2017).

SL showed a significant influence on the root weight of Cu 10^{-2} M treated plants. On the other hand, no significant influence was shown by the shoot weight, root and shoot length, FV/FM ratio and the number of leaves. The significant influence showed on the root weight could be explained by the fact that SL regulates the cytoskeletal dynamics of roots under stress (e.g. Pandya-Kumar *et al.*, 2014; (Liu. Guowei *et al.*, 2017). Additionally in cross talks with other phytohormones such as ethylene, auxin, and cytokinin SL have also been reported to influence root architecture under different types of stress (Ha *et al.*, 2014; Kapulnik and Koltai, 2014). For example, under low-phosphate growth conditions, elevated levels of SL in plants repress shoot branching (Umehara *et al.*, 2010; Kohlen *et al.*, 2011), increase lateral root formation (Ruyter-Spira *et al.*, 2011), and promote root hair density (Mayzlish-Gati *et al.*, 2012). It is without surprise that, mutants defective in the Strigolactone pathway are characterized by an inability to respond to low phosphate levels (Umehara *et al.*, 2008; Kohlen *et al.*, 2011).

4.3.2c. Possible involvement of SL on Mn induced stress in wheat

In this experiment, it was well established that wheat plants exposed to Mn in different concentrations showed varying levels of stress on the different plant parameters measured. Applying varying concentrations of SL showed different effects on different plant parameters of Mn stressed plants.

SL concentrations of 10^{-8} M and 10^{-9} M significantly decreased the root length while the 10^{-9} M treatment considerably decreased the shoot length and root weight of Mn 10^{-3} M treated plants respectively. Treatment of SL 10^{-5} M significantly decreased the shoot weight and the number of leaves of Mn 10^{-3} M treatments. A good explanation for the above observation could be the ability of SL acting in crosstalk

with other phytohormones in the plant e.g. auxin and cytokinin influencing the root and shoot architecture. Previous studies have proven that under optimal growth conditions SLs are also implicated in hormonal crosstalk in the process of root growth, for example, SL inhibits lateral root formation in Arabidopsis (Ruyter-Spira *et al.*, 2011) and encourages root hair elongation (Cui *et al.*, 2018).

SL significantly decreased the FV/FM ratio of Mn 10^{-2} M treated plants at the 10^{-7} M concentration. There was no significant involvement of SL on the root/shoot weight and length and the number of leaves. We propose here that the significant decrease in the FV/FM ratio is due to the ability of SL to influence the hormonal changes in plants under stress, as a result, affects the chloroplast which is the site for most of the photosynthetic processes (Ma *et al.*, 2017).

SL significantly increased the shoot weight of Mn 5.10^{-2} M treated plants at concentrations of SL 10^{-5} M and 10^{-9} M. This influence can be explained by the fact that SL has the ability to invariably influence the shoot architecture enabling the plant to cope with high levels of stress on its metabolic system (Crawford *et al.*, 2010; Domagalska and Leyser, 2011). Additionally even though previously generalizations have been made that SL mostly acts as an inhibitor to shoot growth, recently it has been discovered that SL does not always act as growth inhibitors, for example, it was recently reported that interfascicular cambium development was promoted by SL (Agusti *et al.*, 2012).

4.4. cellular tolerance to HM

When commencing a study with the hope to fully understand the scope at which environmental pressures affect plant growth and development, it is always logical to start from the plant cell (Wei *et al.*, 2016). In plant physiology, the growth and development of the plant are structured to allow the plant cell to enable the plant to respond to current environmental pressures while redirecting the structural context through which other stimuli still to come will be experienced (Dinneny, 2014). In this study, plasmolysis was used as a tool to measure the sensitivity and resilience of the cells of wheat plants to HM ($MnSO_4$ and $CuSO_4$). The primary cell wall of plants is composed of cellulose microfibrils put together in a hydrated matrix of hemicellulose, pectin, and glycoproteins. The moderately firm cellulose microfibrils are considered the main pressure bearing element of the cell wall and provides tensile strength also referred to as the turgid strength (McFarlane, Döring and Persson, 2014). This tensile strength or better still turgidity is lost during plasmolysis as a result of the separation of the living protoplast from the cell wall, due to the loss of water from the cell by the stronger water withdrawing solutions (plasmolytica) for example mannitol as used in this study (Lee-Stadelmann and Stadelmann, 1989). The plasma membrane and tonoplast alterations which occur during plasmolysis are surely one of the most remarkable examples of how to change the surface area to volume ratio of a membrane (without irreparably impairing cell function) (Oparka, 1994).
Leaves were collected from the highest and lowest concentrations of all treatments and from these leaves preferably the upper epidermis, tiny sections were dissected and put into graded concentrations of $CuSO_4$ and $MnSO_4$ respectively. These tiny sections in HM solutions were kept in the dark for 48hrs after which they were observed under the light microscope for viability. The idea behind the cellular tolerance test was to directly induce stress on the cells by placing them in HM solutions and to later observe if the cells remain viable or not. The results from this experiment were needed to be able to tell if the respective treatments had an influence on the ability of the cells to resist HM stress.

In this study we expected the plasmolysis tool to answer the following questions about the cellular tolerance of the treatment groups considered:

- Do the cells of the respective HM treatments show more or less tolerance to the respective HM in solution relative to the C treatment?
- Do the cells of the respective phytohormone treatments show more or less tolerance to the respective HM in solution relative to the C treatment?
- Do the cells of the respective HM/ phytohormone treatments show more or less tolerance to the respective HM in solution relative to the C treatment?

4.4.1a. Cellular tolerance test for Cu

Cu is essential for a plant's metabolism but at the right threshold value above which it becomes toxic and exerts stress on the plant's cells and metabolism as a whole (e.g. Marschner 1995a; Prasad and Strzalka 1999; Yruela 2005). In this study, plants grown in soil treated with Cu 10^{-3} M showed in relation to the C treatment an insignificant decrease or increase in the parameters measured. On the other hand, plants grown on soil treated with Cu 10^{-2} M showed general decreases in all plant parameters measured in comparison with the C treatment. Therefore, it was decided to test the cellular tolerance of Cu 10^{-3} M

and Cu 10^{-2} M because these concentrations both displayed opposite extreme effects to the plant parameters studied.

The cellular tolerance test showed that the cells of the Cu 10^{-2} M and Cu 10^{-3} M treatments were more tolerant of the HM in solution as compared to the cells of the C treatment. A credible explanation as to why the Cu 10^{-2} M and Cu 10^{-3} M treatments showed high levels of tolerance could be that these plants were grown in conditions of high HM and as such their cells must have been acclimatized to this growing condition meanwhile the cells of the C plants were not.

4.4.1b. Cellular tolerance test for Mn

Low Mn levels are necessary for normal nutrition and development of plants; nonetheless, increased levels of Mn concentrations in plant tissues above threshold values can alter a number of important cellular processes, such as enzyme activity, absorption, etc. causing oxidative stress (Ducic and Polle, 2005; Lei, Korpelainen and Li, 2007). In this study, plants grown in soil treated with Mn 10^{-3} M showed in relation to the C an insignificant increase in the parameters measured. On the other hand, plants grown on soil treated with Mn 5.10^{-2} M showed general decreases in all plant parameters measured in comparison with the C treatment. Therefore, it was decided to test the cellular tolerance of these two treatments because these concentrations both displayed opposite extreme effects on the plant parameters studied. The cellular tolerance test showed that the cells of the Mn 10^{-3} M and Mn 5.10^{-2} M treatments were more tolerant of the HM in solution as compared to the cells of the C treatment. A probable explanation to that could be the fact that the Mn treated plants were grown in soil rich in Mn which might have caused acclimatization of their cells thus giving them the added advantage to show more tolerance to $MnSO_4$ in solution relative to the C plants which were grown in soil with no Mn treatments.

4.4.2a. Involvement of JA in the cellular tolerance of Cu treated plants

Generally, the different concentrations of JA applied showed no consistency in the influence exerted on the different plant parameters studied. The roots and FV/FM ratio were the plant parameters most frequently influenced by the application of the phytohormone JA and the concentration 10^{-5} M JA showed significant influences with respect to the C treatments and HM treatments. On the other hand, JA 10^{-9} M treatment rarely but significantly influence the parameters studied. We chose the concentrations 10^{-5} M and 10^{-9} M JA for the cellular tolerance test because they were the highest and lowest concentrations of the JA treatments respectively and thus could give us an idea of the range of concentrations at which JA signaling and transduction works at the level of cellular viability.

First of all, to find out if the application of JA conferred on the plants growing in optimal environmental conditions any kind of cellular tolerance, we compared the JA treatments to the C treatments. The cells of JA 10^{-5} M and 10^{-9} M treatments showed no difference in cellular tolerance when compared to the cells of the C treatment.

secondly, to further investigate if the phytohormone JA had any possible influence on the cellular tolerance of the cells of the Cu/JA treatment to $CuSO_4$ in solution, the cellular tolerance of Cu/JA treatments were compared to the cellular tolerance of the cells of the Cu treatments. The cells of the Cu 10^{-2}/ JA 10^{-5} M treatment showed no difference in cellular tolerance when compared to the cells of the Cu 10^{-2} M treatment meanwhile, the cells of the Cu 10^{-2}/ JA 10^{-9} M treatment were more cellular tolerant as compared to the cells of the Cu 10^{-2} M treatment. Furthermore, the cells of the Cu 10^{-3}/ JA 10^{-5} M treatment showed a significantly low level of cellular tolerance when compared to the cells of the Cu 10^{-3} M treatment. On the other hand, the cells of the Cu 10^{-3} / JA 10^{-9} M treatment were more cellular tolerant when compared to the cells of the Cu 10^{-3} M treatment. A possible explanation for the above observation could be that, plants previously treated with Cu/JA 10^{-9} M might have developed a system that enabled their cells to acclimatize to their growing environment. This could be the result of the activation of JA TFs enabling the expression of JA-responsive genes and JA responses (Huang *et al.*, 2017). These responses may have probably influenced the general structure of the primary cell wall of the plants of Cu/JA 10^{-9} M treatment, either by participating in the formation of cellulose microfibrils pectin, and glycoproteins thus conferring resilience on the cells fortifying the cells against HM. On the other hand, given that phytohormones signaling and transduction are dose sensitive and occur at very low concentrations the treatment of JA 10^{-5} M may not have been the ideal concentration thus leading to the above observations of no significant or reduced cellular tolerance in comparison with the HM treatments.

4.4.2b. Involvement of JA in the cellular tolerance of Mn treated plants

First of all, to find out if the application of JA conferred to the plants growing in optimal environmental conditions any kind of cellular tolerance to $MnSO_4$ in solution, we compared the JA treatment to the C treatments. The JA 10^{-5} M and 10^{-9} M treatment showed a significant decrease in cellular tolerance when

compared to the cells of the C treatment. We suggest here that the application of JA might not have had any effect on the plant's ability to be cellular tolerant because the signaling and transduction pathway was not activated due to the absence of stress at the optimal growth conditions of the plants.

Secondly, to further investigate if the JA had any possible influence on the cellular tolerance of the cells of the Mn/JA treatment to $MnSO_4$ in solution, the cellular tolerance of Mn/JA treatments were compared to the cellular tolerance of the cells of the Mn treatments. The Mn 5.10^{-2}/ JA 10^{-5} M and Mn 5.10^{-2}/ JA 10^{-9} M treatments were more cellular tolerant as compared to the cells of the Mn 5.10^{-2} M treatment respectively. Meanwhile, the cells of the Mn10^{-3}/ JA 10^{-5} M treatment were significantly less tolerant as compared to the cells of the Mn 10^{-3} M treatment. On the other hand, the cells of the Mn10^{-3} / JA 10^{-9} M treatment were more cellular tolerant when compared to the cells of the Mn 10^{-3} M treatment. The treatments with JA 10^{-5} and JA 10^{-9} M both influenced the cellular tolerance of the respective treatments. A good suggestion of an explanation to the above observation could be that in the presence of stress in their growing environment the cells of the plants of these treatments may have perceived the presence of exogenously applied bioactive JA 10^{-5} and 10^{-9} M leading to the JA receptor CORONATINE INSENSITIVE1 (COI1) mediating the ubiquitination and degradation of JAZ proteins via the 26S proteasome (Yan *et al.*, 2007). The resulting activation of TFs may have enabled the expression of JA-responsive genes and JA responses (Huang *et al.*, 2017). These JA responses could possibly show effect in the general structure of the primary cell wall of the plants either participating in the formation of cellulose microfibrils pectin and glycoproteins thus, fortifying the cell wall against HMs.

4.4.3a. Involvement of SL in the cellular tolerance of Cu treated plants

Generally, the different concentrations of SL applied showed no consistency in the influence exerted on the different plant parameters studied. The roots were the plant parameter most frequently influenced by the application of the phytohormone SL and the concentration 10^{-5} M SL also showed significant influences with respect to the C treatments and Hm treatments. On the other hand, SL 10^{-9} M treatment rarely but did significantly influence some parameters studied. We chose the concentrations 10^{-5} M and 10^{-9} M SL for the cellular tolerance test because they were the highest and lowest concentrations of the SL treatments respectively and thus could give us an idea of the range of concentrations at which SL signaling and transduction works at the level of cellular viability.

First of all, to find out if the application of SL conferred on the plants growing in optimal environmental conditions any kind of cellular tolerance to $CuSO_4$ in solution, we compared the JA treatment to the C treatments. The cells of the SL 10^{-5} M treatment were more tolerant as compared to the cells of the C treatment and the cells of the SL 10^{-9} M treatment were significantly more tolerant than the cells of the C treatment. The primary cell wall of plants encompasses cellulose microfibrils embedded in a hydrated matrix of hemicellulose, pectin, and glycoproteins. These components are very important for the cytoskeleton of the cell. SL has been shown to influence the cytoskeleton of root hair and thus elongation of these cells (Pandya-Kumar *et al.*, 2014). We suggest here that the leaf cells of the SL treatments might have developed some form of resilience by the transduction and signaling brought about by the exogenously applied SL thus showing an increase level of cellular tolerance compared to the C treatment.

Secondly, to further investigate if SL had any possible influence on the cellular tolerance of the cells of the Cu/SL treatment to $CuSO_4$ in solution, the cellular tolerance of Cu/SL treatments were compared to the cellular tolerance of the cells of the Cu treatments. The cells of the Cu 10^{-2}/ SL 10^{-5} M treatments showed no difference in cellular tolerance when compared to the cells of the Cu 10^{-2} M treatments, while the cells of the Cu 10^{-2}/ SL 10^{-9} M treatment were significantly more cellular tolerant as compared to the cells of the Cu 10^{-2} M treatment. Furthermore, the cells of the Cu 10^{-3} / SL 10^{-5} M treatment were less tolerant as compared to the cells of the Cu 10^{-3} M treatment meanwhile, the cells of the Cu 10^{-3} / SL 10^{-9} M treatment were significantly more tolerant as compared to the cells of the Cu 10^{-3} M treatment. A likely explanation for the significant increase in the tolerance of the cells treated with SL 10^{-9} M showed could be the possible involvement of SL on the ultrastructure of the cell wall of the plants. We suggest SL application may have contributed to the ultra-structure by participating in the formation of cellulose microfibrils pectin and glycoproteins thus strengthening the cell wall (Pandya-Kumar *et al.*, 2014). Additionally, the significant increases in ROS scavenging and cellular redox homeostasis by SL switching up enzymatic activities of SOD and POD (Anjum *et al.*, 2015) could be a possible reason for the increase in tolerance demonstrated by the SL 10^{-9} M treatments. This activity has been demonstrated in rapeseed whereby exogenous application of SL positively alleviated salinity stress by increasing the scavenging activity of ROS generated by salinity induced stress (Ma *et al.*, 2017).

4.4.3b. Involvement of SL in the cellular tolerance of Mn treated plants

First of all, to find out if the application of SL conferred to the plants growing in optimal environmental conditions any kind of cellular tolerance to $MnSO_4$ in solution, we compared the SL treatments to the C treatments. The cells of the SL 10^{-5} M treatment exhibited a decreased level of cellular tolerance as compared to the cells of the C treatment. On the other hand, the cells of the SL 10^{-9} M treatment showed more tolerance than the cells of the C treatment. A possible explanation for this observation could be that the SL 10^{-5} M may not have been the ideal concentration. On the other hand, the SL 10^{-9} M might have significantly increased cellular tolerance because of the involvement of SL on the cytoskeleton (Pandya-Kumar *et al.*, 2014).

To further investigate if the application of SL had any possible influence on the cellular tolerance of the cells of the Mn/SL treatment to $MnSO_4$ in solution, the cellular tolerance of Mn/SL treatment was compared to the cellular tolerance of the cells of the Mn treatments. The cells of the Mn 5.10^{-2}/ SL 10^{-5} M treatment exhibited less cellular tolerance as compared to the cells of the Mn 5.10^{-2} M treatments respectively. On the other hand, the cells of the Mn 5.10^{-2}/ SL 10^{-9} M treatment were slightly more tolerant as compared to the cells of the Mn 5.10^{-2} M treatment. On the other hand, the cells of the $Mn10^{-3}$/ JA 10^{-5} M treatment showed less cellular tolerance as compared to the cells of the $Mn10^{-3}$ M treatment. On the other hand, the cells of the $Mn10^{-3}$ / JA 10^{-9} M treatment were significantly more tolerant than the cells of the Mn 10^{-3} M treatment. A likely explanation for the significant increase in the tolerance of the cells treated with SL 10^{-9} M could be the possible involvement of SL on the ultrastructure of the cell wall of the plants in their growing environment which could either be by, SL participating in the formation of cellulose microfibrils, pectin and glycoproteins thus strengthening the cell wall (Pandya-Kumar *et al.*, 2014) or the significant increases in ROS scavenging and cellular redox homeostasis by SL switching up enzymatic activities of SOD and POD (Anjum *et al.*, 2015). This activity has been demonstrated in rapeseed by (Ma *et al.*, 2017) wherein the exogenous application of SL positively alleviated salinity stress by increasing the scavenging of ROS generated by salinity.

5. Conclusion

In the last few decades, there has been an increasing awareness of the influence of heavy metals as environmental pollutants. This is principally due to the fact that they can easily be assimilated into biological cycles (Baker and Walker, 1989). With an ever-increasing demand for food, energy, land, and other natural resources, population growth and needs are placing ecosystems under increasing stress levels. An increasing need for bioremediation techniques as well as an increase in agricultural production have both begged for current research to be tailored towards discovering sustainable techniques to solve this problem. For example, the current input of huge amounts of Pi into the soil to increase agricultural yield by farmers is not a sustainable practice coupled to the fact that current methods used nowadays to remediate HM polluted soils are either very expensive or ineffective (Marques, Rangel and Castro, 2009). Thus, finding eco-friendly, sustainable and economical methods to tackle these problems are trivial. Exogenous application of phytohormones has proven to positively influence certain key processes in plants such as transpiration rate, cell division, phosphorus metabolism and assimilation etc. these are processes that are key to plant growth and development. The application of the use of phytohormones on plants growing on HM soil can create new avenues for agricultural production and plants for bioremediation (Sytar *et al.*, 2019). Additionally, this will not only contribute to knowledge on plant biofortification but can also lead to bioremediation. Finding ways to enable agricultural plants to grow in suboptimal environmental conditions which will not only increase food production but remediate the environment was the purpose of this study.
In this study, the effects of HMs, Cu, and Mn and the possible involvement of phytohormones, JA and SL on the growth improvement and stress tolerance of wheat a very important agricultural plant were investigated. Wheat plants were subjected to heavy metal stress in a two-phase experimental design of approximately five and four weeks respectively. To fortify the plants and enable them to cope with the abiotic stress induced by the HMs in the soil, the plant's leaves were sprayed with graded concentrations of phytohormones, JA for the first phase, and SL for the second phase of the experiments of this study. The aim of this study was first to assess the effects of HMs, Cu, and Mn on different plant parameters of wheat, secondly to investigate the possible involvement of phytohormones on the growth performance of wheat and thirdly to investigate the conference of HM stress tolerance on wheat by applying the respective phytohormones.

The level of abiotic stress on the plants increased synchronously with the increase in the concentration of the respective HMs applied to the soil. Because of the ability of these HM elements to initiate oxidative damage and interfere with important cellular processes such as photosynthesis, pigment synthesis, etc. it is important their concentration be regulated within the cell. They are essential and usually bind to proteins nevertheless, they have the ability to strongly inhibit plant growth and development especially above threshold values (Van Assche and Clijsters, 1990; Yrule, 2005). In comparison to the C, there were insignificant decreases in the plant parameters of the lowest concentrations of the respective HMs applied and on the other hand, a significant decrease in all parameters of the highest concentrations of HMs applied. This was in line with previous studies that have well proven that when plants are exposed to HMs, they can show a range of effects from growth-promoting to growth-inhibiting. The most significant effects of the respective HMs were shown on the plant's root length and weight. This was expected given that the roots are the first to get in contact with the HMs in soil and thus must acclimatize to prevent uptake into the shoots which are the most delicate parts of the plant. Previous studies have well demonstrated this phenomenon and it is referred to as the soil root and shoot barrier (e.g. Cunningham, 1995).

Generally, the various concentrations of phytohormones JA and SL applied did not show consistency in the effects showed on the different parameters of the plants. Nevertheless, JA and SL frequently significantly influenced root parameters.

Firstly, all concentrations of MeJA applied inconsistently affected all plant parameters studied here but generally speaking, JA 10^{-5} M and 10^{-6} M were the concentrations that frequently showed significant influences on the various plant parameters studied. The root length and the FV/FM ratio here were the plant parameters that were most frequently influenced by the application of JA acid.

secondly, all concentrations of SL applied inconsistently affected all plant parameters studied here but generally speaking, for the treatment with SL, the 10^{-5} M concentration was the concentration that most frequently showed significant influences on the plant parameters studied. The root weight was the plant parameter most frequently influenced by the application of SL.

Even though in recent decades, the JA and SL signaling pathways have been extensively investigated, the current understanding of their role in different environmental stresses is still limited. This is due to the complex networks and crosstalk between multiple stresses and multiple signaling pathways in different plant species. So far, the molecular mechanism of JA and SL signaling in stress responses remains elusive. Compared with well-studied components, the studied components in plant hormone signaling pathways are incomplete. The plant hormone signaling network is complex and changeable and also plant-specific. Even though in the last decade a huge volume of research has been carried out and has successfully provided a global analysis of gene expression and protein spectrum changes, the data still fails in a great number of ways. One important failure is the inability of this data to give a full understanding of the dynamic, spatial and temporal progressions of plant hormone signaling networks in the stability between plant growth and defense resistance. Moreover, most experiments are carried out in the lab and data gotten from the lab is in many ways different from field data. Therefore, it is necessary to detailly analyze plant hormone networks during the whole developmental stage of the crop in the field. This will provide real-life data that can be reliable for applicability. Therefore, we have to state that, the current understanding remains limited compared with unknown questions. Further research will offer a new understanding of the development of plant hormones for agricultural production of wheat focusing on improving stress resistance, crop quality and bioremediation.

We, therefore, conclude that the intensity of the effects of HMs Mn and Cu on wheat was dependent on the concentration of HM in the soil. Secondly, the application of the phytohormones JA and SL showed significant changes even though not consistent in the plant parameters measured and thus were implicated in the growth performance and the HM stress tolerance of wheat. Further research on this to define the exact concentrations of the phytohormones to be applied and the best mode of application will go a long way to produce significant effects on the yield and stress tolerance of wheat.

6. References

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7. Supplementary information

7.1. All data for the first phase of this study experimenting with Jasmonic acid

Treatment	RL/cm	SL/cm	RW/g	SW/g	FV/FM	Num of Leaves
C	12,30	37,20	0,258	0,568	0,793	6,40
C	13,50	41,00	0,291	0,63	0,788	6,00
C	13,00	36,80	0,402	0,77	0,785	4,60
JA5	9,88	39,88	0,236	0,566	0,785	5,75
JA5	9,80	35,20	0,235	0,545	0,781	4,60
JA5	12,00	37,75	0,226	0,513	0,799	6,50
JA6	12,25	45,25	0,316	0,742	0,784	5,75
JA6	11,20	44,70	0,269	0,7	0,781	5,00
JA6	9,38	43,25	0,23	0,64	0,781	5,25
JA7	11,40	40,90	0,234	0,655	0,815	5,40
JA7	10,00	43,75	0,287	0,696	0,813	5,00
JA7	12,90	41,40	0,283	0,825	0,777	5,20
JA8	13,00	46,38	0,258	0,685	0,761	4,75
JA8	12,20	41,10	0,25	0,675	0,75	5,00
JA8	11,13	41,75	0,227	0,566	0,799	4,75
JA9	12,30	37,50	0,223	0,182	0,77	4,60
JA9	11,70	35,70	0,271	0,639	0,795	5,00
JA9	12,60	38,70	0,294	0,696	0,805	5,00
Cu-3	11,00	39,63	0,237	0,598	0,801	5,50
Cu-3	11,13	43,75	0,226	0,623	0,802	5,50
Cu-3	9,25	41,88	0,203	0,535	0,797	5,00
Cu-3_JA-5	9,60	37,8	0,246	0,602	0,811	5,2
Cu-3_JA-5	10,20	42,1	0,305	0,723	0,794	5,8
Cu-3_JA-5	9,25	43,25	0,195	0,545	0,815	5,5
Cu-3_JA-6	9,50	39,4	0,275	0,617	0,83	5,2
Cu-3_JA-6	11,00	40,8	0,29	0,721	0,825	5
Cu-3_JA-6	11,50	47	0,216	0,661	0,838	5,75
Cu-3_JA-7	11,00	40,6	0,284	0,707	0,789	5,2
Cu-3_JA-7	10,40	41,4	0,279	0,593	0,771	5,2
Cu-3_JA-7	9,20	40,2	0,211	0,518	0,807	5,80
Cu-3_JA-8	10,10	41,3	0,31	0,763	0,786	5,80
Cu-3_JA-8	10,80	40,4	0,25	0,726	0,793	4,60
Cu-3_JA-8	10,30	40,6	0,237	0,62	0,797	5,40

Cu-3_JA-9	11,70	37,4	0,202	0,545	0,812	5,40
Cu-3_JA-9	13,00	39,125	0,254	0,583	0,778	9,25
Cu-3_JA-9	10,10	33	0,18	0,525	0,789	5,20
Cu 5(-3)	7,00	36,30	0,14	0,576	0,785	5,50
Cu 5(-3)	5,90	25,90	0,089	0,302	0,745	5,50
Cu 5(-3)	7,63	32,88	0,116	0,429	0,786	6,25
Cu 5(-3)_JA-5	7,00	26,63	0,085	0,26	0,75	6,50
Cu 5(-3)_JA-5	6,30	32,80	0,146	0,443	0,563	6,20
Cu 5(-3)_JA-5	7,60	31,10	0,138	0,336	0,593	6,20
Cu 5(-3)_JA-6	5,20	24,40	0,116	0,379	0,668	5,80
Cu 5(-3)_JA-6	5,50	26,30	0,69	0,326	0,764	5,20
Cu 5(-3)_JA-6	5,00	29,50	0,066	0,272	0,718	5,75
Cu 5(-3)_JA-7	6,63	27,13	0,09	0,275	0,765	6,0
Cu 5(-3)_JA-7	7,38	29,63	0,097	0,328	0,702	6,8
Cu 5(-3)_JA-7	7,40	32,20	0,198	0,453	0,735	6,4
Cu 5(-3)_JA-8	9,00	35,88	0,087	0,403	0,802	5,8
Cu 5(-3)_JA-8	7,90	37,40	0,232	0,581	0,689	6,0
Cu 5(-3)_JA-8	9,63	36,88	0,163	0,441	0,672	6,0
Cu 5(-3)_JA-9	10,33	28,17	0,075	0,201	0,805	7,3
Cu 5(-3)_JA-9	9,00	31,67	0,119	0,36	0,67	8,0
Cu 5(-3)_JA-9	8,40	32,20	0,125	0,461	0,578	6,6
Cu-2	0,50	17,50	0,069	0,202	0,716	6,00
Cu-2	5,75	23,25	0,092	0,259	0,702	6,00
Cu-2	0,83	15,20	0,037	0,143	0,717	5,50
Cu-2_JA-5	3,00	18,88	0,069	0,174	0,776	5,75
Cu-2_JA-5	2,88	17,63	0,059	0,139	0,701	6,50
Cu-2_JA-5	2,60	18,50	0,070	0,227	0,697	5,80
Cu-2_JA-6	0,88	12,13	0,052	0,094	0,794	5,50
Cu-2_JA-6	0,50	11,00	0,047	0,11	0,729	5,40
Cu-2_JA-6	0,70	14,70	0,081	0,14	0,565	5,00
Cu-2_JA-7	0,50	11,00	0,02	0,059	0,6	4,67
Cu-2_JA-7	0,50	9,90	0,029	0,089	0,773	5,20
Cu-2_JA-7	0,50	10,20	0,047	0,093	0,761	5,00
Cu-2_JA-8	1,10	18,90	0,076	0,221	0,811	6,20
Cu-2_JA-8	1,13	20,50	0,079	0,204	0,739	7,00
Cu-2_JA-8	4,60	29,90	0,131	0,433	0,78	8,20
Cu-2_JA-9	1,67	14,33	0,039	0,098	0,508	6,00
Cu-2_JA-9	1,25	14,88	0,057	0,126	0,793	6,00
Cu-2_JA-9	1,50	14,63	0,049	0,114	0,783	5,50

Treatment	RL/cm	SL/cm	RW/g	SWG	FV/FM	Num of Leaves
Mn-3	14,13	35,60	0,27	0,79	0,802	5,40
Mn-3	11,63	40,75	0,22	0,63	0,811	6,00
Mn-3	11,63	40,38	0,29	0,78	0,809	6,50
Mn-3_JA-5	13,54	38,20	0,18	0,64	0,79	5,60
Mn-3_JA-5	15,75	38,50	0,19	0,56	0,776	5,00

Mn-3_JA-5	14,13	41,88	0,21	0,61	0,859	5,50
Mn-3_JA-6	12,10	41,90	0,18	0,66	0,824	5,40
Mn-3_JA-6	14,10	39,40	0,21	0,77	0,766	4,80
Mn-3_JA-6	13,40	46,20	0,24	0,80	0,796	5,40
Mn-3_JA-7	15,00	50,67	0,22	0,63	0,757	6,00
Mn-3_JA-7	13,10	44,80	0,23	0,80	0,807	6,00
Mn-3_JA-7	14,00	47,20	0,26	0,95	0,792	5,40
Mn-3_JA-8	12,13	46,00	0,19	0,70	0,791	5,00
Mn-3_JA-8	11,75	43,75	0,23	0,65	0,803	4,75
Mn-3_JA-8	10,60	41,60	0,17	0,66	0,8	5,00
Mn-3_JA-9	13,67	48,00	0,17	0,61	0,799	6,00
Mn-3_JA-9	11,75	38,50	0,19	0,56	0,818	5,25
Mn-3_JA-9	12,20	43,40	0,23	0,64	0,788	5,20
Mn-2	8,00	32,50	0,07	0,74	0,797	5,40
Mn-2	10,67	36,00	0,11	0,77	0,757	6,33
Mn-2	12,00	38,80	0,26	0,65	0,743	5,60
Mn-2_JA-5	14,63	33,38	0,11	0,64	0,809	5,25
Mn-2_JA-5	9,40	27,10	0,07	0,54	0,802	4,60
Mn-2_JA-5	13,25	35,50	0,15	0,68	0,799	6,00
Mn-2_JA-6	9,83	35,00	0,05	0,41	0,795	5,33
Mn-2_JA-6	10,80	25,20	0,06	0,56	0,801	5,40
Mn-2_JA-6	10,25	31,33	0,09	0,67	0,78	6,25
Mn-2_JA-7	10,70	29,00	0,09	0,68	0,79	5,40
Mn-2_JA-7	11,70	33,90	0,20	0,88	0,823	6,60
Mn-2_JA-7	11,67	32,50	0,07	0,56	0,792	7,00
Mn-2_JA-8	15,50	37,40	0,28	0,84	0,803	5,20
Mn-2_JA-8	10,40	29,30	0,09	0,69	0,772	6,00
Mn-2_JA-8	11,60	35,10	0,18	0,71	0,799	6,00
Mn-2_JA-9	15,40	32,50	0,21	0,70	0,8	5,40

Mn-2_JA-9	15,25	38,50	0,17	0,68	0,803	8,00
Mn-2_JA-9	17,63	39,25	0,28	0,69	0,791	5,50
Mn 5(-2)	6,25	19,30	0,02	0,24	0,679	3,60
Mn 5(-2)	5,88	22,60	0,03	0,29	0,757	3,60
Mn 5(-2)	6,00	21,00	0,02	0,24	0,694	3,25
Mn 5(-2)_JA-5	9,10	20,40	0,05	0,31	0,37	3,60
Mn 5(-2)_JA-5	7,50	18,50	0,03	0,20	0,637	3,75
Mn 5(-2)_JA-5	10,30	20,10	0,04	0,25	0,641	4,20
Mn 5(-2)_JA-6	8,24	20,80	0,04	0,27	0,689	3,00
Mn 5(-2)_JA-6	10,50	22,75	0,03	0,18	0,747	4,50
Mn 5(-2)_JA-6	7,40	17,20	0,05	0,31	0,718	3,60
Mn 5(-2)_JA-7	11,00	20,30	0,05	0,38	0,727	3,80
Mn 5(-2)_JA-7	9,50	24,13	0,03	0,28	0,758	3,75
Mn 5(-2)_JA-7	9,50	21,00	0,03	0,23	0,742	4,50
Mn 5(-2)_JA-8	10,13	21,50	0,04	0,27	0,692	4,00
Mn 5(-2)_JA-8	10,70	21,00	0,04	0,26	0,726	4,20
Mn 5(-2)_JA-8	11,20	23,00	0,05	0,35	0,596	4,20
Mn 5(-2)_JA-9	11,00	19,90	0,04	0,27	0,802	4,20
Mn 5(-2)_JA-9	9,40	19,70	0,05	0,27	0,426	3,20
Mn 5(-2)_JA-9	8,50	14,83	0,03	0,14	0,645	3,33

7.2. All data for second phase of this study experimenting with Strigolactone

Treatment	RL/cm	SL/cm	RW/g	SW/g	FV/FM	Num of Leaves
C	13,7	39,2	0,081	0,33	0,793	5
C	17,3	36,4	0,062	0,248	0,807	5
C	11,7	36,4	0,068	0,272	0,769	4,8
SL5	12,1	36	0,083	0,312	0,804	5,2
SL5	13,6	34	0,04	0,271	0,771	4,8
SL5	15,6	33,6	0,076	0,301	0,77	5
SL6	17,2	33,4	0,055	0,324	0,776	5,4
SL6	15,4	37,5	0,06	0,32	0,758	5
SL6	9,9	36,7	0,056	0,336	0,745	4,8
SL7	9,4	35,2	0,029	0,252	0,787	4,8
SL7	14,2	37,5	0,048	0,266	0,775	5

SL7	9,9	38,8	0,048	0,297	0,723	4,8
SL8	12,6	39,2	0,078	0,41	0,783	5,2
SL8	9,7	35,6	0,055	0,3	0,751	5
SL8	12,2	37,3	0,064	0,378	0,737	5
SL9	15	35,3	0,039	0,306	0,798	4,8
SL9	8,8	35,2	0,081	0,329	0,785	5
SL9	11,1	37,7	0,054	0,307	0,81	5
Cu-3	12,1	32,4	0,069	0,251	0,769	4,8
Cu-3	10,3	33,3	0,08	0,251	0,873	5
Cu-3	12,9	34,3	0,076	0,246	0,741	4,2
Cu-3_SL-5	11	32,3	0,07	0,279	0,758	5
Cu-3_SL-5	9,9	30,6	0,061	0,238	0,786	5
Cu-3_SL-5	8,6	27,8	0,046	0,173	0,81	4,6
Cu-3_SL-6	10,6	33,1	0,061	0,288	0,793	5
Cu-3_SL-6	7,63	32,63	0,023	0,15	0,78	4,25
Cu-3_SL-6	9,1	34,9	0,04	0,211	0,804	4,6
Cu-3_SL-7	9,7	34,2	0,096	0,268	0,786	5,4
Cu-3_SL-7	9,4	35	0,062	0,26	0,799	5,4
Cu-3_SL-7	11,67	31,67	0,044	0,165	0,8	5
Cu-3_SL-8	8	34,4	0,045	0,182	0,798	5
Cu-3_SL-8	13,6	31,3	0,057	0,264	0,784	5
Cu-3_SL-8	12,8	34,67	0,041	0,165	0,798	5,333
Cu-3_SL-9	10,2	30,4	0,071	0,246	0,793	4,8
Cu-3_SL-9	10	31,5	0,067	0,238	0,778	5
Cu-3_SL-9	10,3	30,1	0,066	0,213	0,773	4,8
Cu 5(-3)	5,4	29	0,044	0,177	0,755	5,2
Cu 5(-3)	3,6	30,1	0,05	0,205	0,785	5
Cu 5(-3)	4,1	25,2	0,05	0,223	0,753	4,8
Cu 5(-3)_SL-5	3,7	24,4	0,026	0,166	0,613	4,8
Cu 5(-3)_SL-5	3,625	23,375	0,018	0,092	0,736	4,5
Cu 5(-3)_SL-5	4,5	32,875	0,028	0,197	0,758	5
Cu 5(-3)_SL-6	3,625	28,5	0,017	0,168	0,788	4,75
Cu 5(-3)_SL-6	3	27,5	0,022	0,146	0,731	4,5
Cu 5(-3)_SL-6	4,2	30,4	0,017	0,226	0,788	4,6
Cu 5(-3)_SL-7	4,3	27,8	0,061	0,217	0,767	4,8
Cu 5(-3)_SL-7	4,6	30,1	0,059	0,205	0,758	4,6
Cu 5(-3)_SL-7	3,7	31,1	0,054	0,214	0,801	4,8
Cu 5(-3)_SL-8	4,14	29,1	0,042	0,187	0,762	4,6
Cu 5(-3)_SL-8	2,64	29,7	0,041	0,191	0,785	4,4
Cu 5(-3)_SL-8	2,8	30,8	0,045	0,193	0,769	4,2
Cu 5(-3)_SL-9	4,3	33,4	0,076	0,238	0,786	5,2
Cu 5(-3)_SL-9	4,4	26,6	0,041	0,161	0,799	4,4
Cu 5(-3)_SL-9	4,7	31	0,05	0,215	0,78	5
Cu-2	0,36	16,1	0,015	0,101	0,783	3,8
Cu-2	0,7	23,1	0,04	0,157	0,712	4,8
Cu-2	0,525	18,25	0,024	0,107	0,795	4,25
Cu-2_SL-5	0,84	18,9	0,049	0,122	0,797	4,2
Cu-2_SL-5	0,5	18,3	0,049	0,127	0,796	4,2
Cu-2_SL-5	0,5	17,5	0,05	0,109	0,776	3,6
Cu-2_SL-6	1,06	21,2	0,04	0,157	0,784	4,2

Cu-2_SL-6	0,5	19,8	0,047	0,133	0,763	4
Cu-2_SL-6	0,525	23,75	0,036	0,129	0,775	4,25
Cu-2_SL-7	0,32	17,8	0,004	0,124	0,79	4
Cu-2_SL-7	0,42	20,5	0,039	0,128	0,782	4
Cu-2_SL-7	0,34	17,3	0,007	0,101	0,781	4
Cu-2_SL-8	1,45	23,375	0,045	0,155	0,743	4,5
Cu-2_SL-8	0,567	19,5	0,019	0,081	0,778	4
Cu-2_SL-8	0,68	20,5	0,037	0,139	0,759	4,2
Cu-2_SL-9	0,3	18	0,023	0,114	0,8	4,2
Cu-2_SL-9	0,38	17,9	0,024	0,12	0,796	4
Cu-2_SL-9	0,2	19	0,041	0,23	0,781	4,2

Treatment	RL/cm	SL/cm	RW/g	SW/g	FV/FM	Num of Leaves
Mn-3	15,333	39,667	0,071	0,263	0,761	5
Mn-3	14,908	41,9	0,059	0,357	0,757	5
Mn-3	13	39,9	0,089	0,393	0,804	5
Mn-3_SL-5	13	31,33	0,042	0,14	0,752	3,67
Mn-3_SL-5	13	34,33	0,042	0,195	0,8	4,67
Mn-3_SL-5	13,9	35,8	0,078	0,285	0,775	5
Mn-3_SL-6	13,6	39,1	0,092	0,448	0,783	5,2
Mn-3_SL-6	12,2	35,7	0,064	0,34	0,79	4,8
Mn-3_SL-6	10,8	37,4	0,066	0,338	0,798	4,8
Mn-3_SL-7	9,8	38,9	0,06	0,271	0,801	4,8
Mn-3_SL-7	13,2	35,9	0,074	0,356	0,781	4,8
Mn-3_SL-7	14,8	38,2	0,06	0,285	0,768	4,8
Mn-3_SL-8	10,8	36,4	0,069	0,285	0,774	4,4
Mn-3_SL-8	10,167	37	0,06	0,209	0,773	5
Mn-3_SL-8	12,1	37,5	0,081	0,247	0,779	5
Mn-3_SL-9	8,5	37,5	0,042	0,25	0,757	4,75
Mn-3_SL-9	13,2	38,2	0,064	0,361	0,758	5
Mn-3_SL-9	10,833	40,33	0,039	0,239	0,772	5
Mn-2	11,5	39	0,037	0,296	0,781	5
Mn-2	13,5	37,9	0,049	0,372	0,746	4,8
Mn-2	13	35,9	0,07	0,355	0,772	5,2
Mn-2_SL-5	9,8	33,3	0,031	0,268	0,799	4,4
Mn-2_SL-5	12,6	36,1	0,039	0,351	0,783	5
Mn-2_SL-5	11,6	37,3	0,058	0,359	0,772	5
Mn-2_SL-6	11,6	35,1	0,044	0,328	0,784	4,8
Mn-2_SL-6	8,8	36,1	0,04	0,332	0,793	4,8
Mn-2_SL-6	10,167	40,5	0,023	0,25	0,797	5
Mn-2_SL-7	15,1	36,9	0,066	0,372	0,7	5
Mn-2_SL-7	11,6	32,7	0,047	0,316	0,748	5
Mn-2_SL-7	13	36,2	0,055	0,329	0,743	5
Mn-2_SL-8	13,5	41,3	0,082	0,403	0,788	5
Mn-2_SL-8	10,3	34,3	0,058	0,351	0,781	5,2
Mn-2_SL-8	9,36	38,2	0,063	0,357	0,773	5
Mn-2_SL-9	9,6	39,2	0,052	0,374	0,801	5
Mn-2_SL-9	10,6	36	0,051	0,351	0,798	5,2
Mn-2_SL-9	13,333	38	0,027	0,192	0,761	5

Mn 5(-2)	9,5	20,2	0,035	0,179	0,793	2,6
Mn 5(-2)	11,333	20,5	0,025	0,136	0,7	3,333
Mn 5(-2)	9,667	18,333	0,021	0,105	0,78	3
Mn 5(-2)_SL-5	7,8	22,8	0,038	0,214	0,804	3,6
Mn 5(-2)_SL-5	9	22	0,043	0,21	0,786	3,2
Mn 5(-2)_SL-5	9,3	23,2	0,041	0,211	0,791	3,4
Mn 5(-2)_SL-6	9,9	20	0,029	0,168	0,786	2,8
Mn 5(-2)_SL-6	9,7	20,4	0,05	0,174	0,786	2,8
Mn 5(-2)_SL-6	9,875	22,625	0,039	0,178	0,782	2,75
Mn 5(-2)_SL-7	7,667	21,167	0,029	0,107	0,784	3,667
Mn 5(-2)_SL-7	8,5	18,2	0,044	0,134	0,771	2,8
Mn 5(-2)_SL-7	9,5	17,25	0,038	0,208	0,801	2
Mn 5(-2)_SL-8	9,2	18,6	0,044	0,189	0,781	2,6
Mn 5(-2)_SL-8	9	20,5	0,038	0,191	0,782	2,75
Mn 5(-2)_SL-8	9,6	18,6	0,03	0,176	0,79	2
Mn 5(-2)_SL-9	8,2	22,7	0,021	0,178	0,776	3,2
Mn 5(-2)_SL-9	8,5	21,7	0,041	0,215	0,726	3,4
Mn 5(-2)_SL-9	7,6	16,9	0,043	0,26	0,795	2,8

RL/cm: Root Length in cm

SL/cm: Shoot Length in cm

RW/g: Root Weight in grams

SW/g: Shoot Weight in grams

FV/FM: FV/FM ratio

NUM of leaves: The number of leaves

SL: Strigolactone (GR24)

JA: Jasmonic acid (MeJA)