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IMPACT OF SALT STRESS AND INDOLE ACETIC ACID REGULATION ON THE MORPHO-ANATOMICAL ATTRIBUTES OF AVENA SATIVA

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ABSTRACT

Salt stress limits crop productivity by negatively impacting plant growth. *Avena sativa* (oat) is particularly vulnerable to salinity, but phytohormones like Indole Acetic Acid (IAA) may help mitigate these effects. This study examined the impact of NaCl-induced salt stress on *Avena sativa* and evaluated the role of exogenously applied IAA in alleviating stress-related

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damage. Plants were subjected to varying NaCl concentrations, with and without IAA treatment. Morphological and anatomical traits, including plant height, biomass, stomatal density, and vascular structure, were analyzed. Salt stress significantly reduced growth parameters, but IAA application improved root development, stomatal density, and vascular robustness. The response to IAA was dose-dependent, with optimal benefits at specific concentrations. IAA mitigates the adverse effects of salt stress on *Avena sativa*, enhancing growth and anatomical structure. These findings highlight its potential as a stress regulator, warranting further molecular studies for crop improvement.

KEYWORDS: Avena sativa, salt stress, Indole Acetic Acid, plant growth, anatomical adaptation

1. Introduction

Salinity issues in soil and water pose a significant threat to the world's ability to produce food. A better understanding of how soil water balances about evapotranspiration (ET) are impacted by the temporal and spatial dynamics of salinity and the creation of effective irrigation schedules and techniques are two strategies to deal with Salinity. Both in a constant state Salinity issues in soil and water pose a significant threat to the world's ability to produce food. A deeper comprehension of the effects of Salinity's temporal and geographical dynamics on soil water balances about evapotranspiration (ET) and the creation of effective irrigation schedules and techniques are two strategies to deal with Salinity(Minhas, Ramos, Ben-Gal, & Pereira, 2020). One of the main abiotic barriers to world food production is soil and water Salinity, along with related issues. These challenges are especially important in semi-arid and arid regions. Using Salina groundwater for irrigated agriculture is a frequent practice in areas where water is scarce(Steane, Chowdhury, & Foot, 1992). The world's coastal zones are home to a sizable tract of low-yielding, Salinized land that has been abandoned. Soil Salinity poses a significant and persistent challenge to agricultural productivity by impeding plant growth and reducing crop yield(Majeed & Muhammad, 2019). One workable solution to this issue is to increase plant tolerance to Salt. The physiological and biochemical reactions of plants to Salt stress have

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gained attention recently because they may shed light on potential genetic modifications that could make plants more tolerant. It is well-recognized that ion toxicity, oxidative stress, and osmotic stress are the main causes of the detrimental effects of soil Salinity on plants(Yadav, Irfan, Ahmad, & Hayat, 2011). In recent years, there has been significant advancement in our understanding of the molecular and physiological mechanisms underlying plant tolerance to Salt. However applying current knowledge to agronomy research in a field setting is difficult(Yan, Craddock, Zuo, Zang, & Milham, 2013). Salinity currently affects more than 800 million hectares of land worldwide ("OECD-FAO Agricultural Outlook, 2008-2017," 2009)

The Salina land covers all of the continents, including Africa, Asia, Australasia, and America, and makes up more than 6% of the planet's geographical surface. Unlike animals, plants are unable to flee unfavorable situations and must instead undergo several physiological processes to adjust to them(Weiner, 1992). Plant biomass was frequently reduced as a result of environmental stress, particularly Salt stress. It is a conventional and thorough indicator of the detrimental impact that unfavorable environmental conditions have on plants. Plant biomass, however, is not a sensitive metric because the long-term effects of a bad environment induce a decline in biomass accumulation. Plant development and agricultural production are strongly correlated with photosynthesis, which is also extremely vulnerable to environmental stress (Yan et al., 2013).

Global agriculture continues to face a major challenge in feeding the world's rising population, which is now growing at a pace of roughly 1.05% each year (World Bank Open Data, n.d.). The growth, production, productivity, and quality of food are all significantly impacted by a wide range of biotic and abiotic stresses(Gopalakrishnan et al., 2018). Biologic stressors include infections or damages brought on by different pathogens or pests. Drought, Salinity, temperature, heavy metals, and other organic pollutants are examples of abiotic stressors. Soil salinization is the most harmful of all abiotic stressors(Daliakopoulos et al., 2016). Salinity stress also adds to oxidative stress by raising the production of reactive oxygen species (ROS), which damage proteins, lipids, nucleic acids (DNA, RNA), and cell

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membranes. ROS also can cause programmed cell death. Furthermore, salinity induces an excessive accumulation of Na+ and Cl– ions, which results in hypertonic stress (Zhang & Zhu, 2018). Soil Salinity can be roughly categorized into two groups: naturally occurring and man-made. The most significant factors include natural processes like rainfall, its subsequent evaporation, and the soil's derivation from a Salina parent material. When significant quantities of Salt are present due to naturally occurring high-salt mineral formations, groundwater irrigation also results in Salina conditions. Phreatophytes that grow alongside canals under water stress situations increase the Salinity levels by eating the water and transferring the Salts to the remaining water that is utilized for irrigation. These plants grow alongside irrigated areas' canals and drains (Carter, 1975). One effect of Salinity on the soil is surface crusting. During the early growing seasons, especially when the soil is devoid of crop canopy cover, the process of crusting causes hard, white layers to form on the soil's surface. Numerous causes contribute to surface crusting. Physical and chemical dispersion are the two categories based on the dispersion technique. Raindrops and irrigation water influence the soil, breaking down weak soil aggregates to form a surface seal that is referred to as crusting(Lawrence et al., 2007).

Oat (Ahmad, Dar, & Habib, 2014) is a member of the cereal family and is used as a cereal fodder crop. Unlike other cereals and pseudocereals, the oat (*Avena sativa*), sometimes referred to as the common oat, is a kind of cereal grain that is farmed for its seed, which goes by the same name (typically in the plural)(A. Capurso, Crepaldi, & Capurso, 2018). The herbaceous plant *Avena sativa* L. is a biennial member of the Poaceae family(Kim et al., 2021). It resembles barley in form and comes in a variety of varieties, such as black, red, yellow, and white oats(Moral et al., 2021). The conventional wisdom holds that oats originated as a weed of the principal cereal domesticates, became a subsidiary crop, and then expanded westward into cooler, wetter climates that were ideal for oat growth. This finally led to the domestication of oats in parts of the Middle East and Europe (Burger, 2008).

The cultivation season of oats is similar to wheat crops. Seasonal flowering behavior, or phenology, is a key contributor to the success of oats as a crop. As a species, oat is a

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vernalization-responsive long-day plant that flowers after winter as days lengthen in spring(Bagheri et al., 2022). The cooler temperatures and lower humidity levels during this period create favorable conditions for oat cultivation, particularly in the northern regions of the country where winters are more pronounced(Dheeravathu, Singh, Srinivasan, & Yadav, 2022). Compared to other small-grain cereal crops, oats are more suited to a variety of soil types and can even function better in acidic soils. They can be susceptible to hot, dry conditions from the time of head emergence to maturity because they are primarily grown in cold, damp regions (Mahtab Ahmad et al., 2014).Among grains produced worldwide, they rank sixth, behind wheat, maize, rice, barley, and sorghum(Demirkesen & Bayhan, 2019). Oats have numerous uses in foods; most commonly, they are rolled or crushed into oatmeal, or ground into fine oat flour(Ahmad *et al.*, 2014). Oatmeal is chiefly eaten as porridge, but may also be used in a variety of baked goods, such as oatcakes, oatmeal cookies, and oat bread (Pallavi & Ravi Kumar, 2021). Oats are also an ingredient in many cold cereals, in particular muesli and granola (Costa-Font and Revoredo-Giha, 2019). Oats are mostly composed of globulin, which makes up about 70–80% of their protein composition. Prolamin is present in smaller amounts(Klose & Arendt, 2012). Compared to other cereal crops, oats have 5–12% more fat (Maheshwari *et al.*, 2019). Oats contain around 95% fat in the form of palmitic, oleic, and linoleic acids, and 75–80% of that fat is unsaturated fatty acids. These unsaturated fatty acids are linked to several advantageous physiological characteristics, including antioxidant activity and the prevention of dementia(M. Capurso et al., 2021). Unsaturated fatty acids have recently been demonstrated to reduce blood cholesterol, which has raised interest in oats as a functional diet (Shvachko et al., 2021).

In Pakistan, oats are more widely tillered and yield many cuts than wheat and barley(Murhekar et al., 2021), with high yield and higher nutritional content. Hay, silage chaff, haylage, feed, fodder, bedding straw, and human food are all made from oats. Mostly, they were pounded into ideal oat flour or rolled, crushed, or even made into oatmeal. Although oatmeal is generally consumed as porridge, it may also be used as a raw material to make food, cosmetics, and health care items. Baked goods such as oatbread, oatmeal

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cookies, and oatcakes can also be made with them (Carlsson, Schimmack, Williams, & Bürkner, 2017).

A sufficient supply of feed that is delivered on schedule is essential to the health and prosperity of livestock production. The primary and most affordable source for meeting livestock's nutritional demands is green forages. One main factor attributed to low animal performance for milk and meat production is the inadequate availability of feed(Rana, Ardichvili, & Tkachenko, 2014). With an 11.53% GDP contribution, livestock is Pakistan's most important agricultural sector. However, a sizable fraction of animals are malnourished. On the other hand, animals that are fed low-quality fodder continuously and over an extended period become malnourished. With an average yield of 22.38 t ha−1, we are currently producing 55.06 million tonnes of fresh feed yearly from 2.46 million hectares (Bilal et al., 2017).

Despite its importance, it is being affected by abiotic factors such as cold, temperature(Saeed, Muneer, Haq, & Akram, 2022), drought(Wahab et al., 2022), heavy metals(Joanito et al., 2022), Salt concentration(Khan et al., 2022), and nutrient deficiency (Adnan et al., 2022)drastically affect crop yield and productivity. Many abiotic and biotic factors are responsible for minimum production in the world(Al-Mamun et al., 2021). Owing to these issues, heavy metal contamination has become a new source of worry for soil agronomists, as it is negatively impacting crop productivity and agroecosystems. Once deposited over specified allowable levels, the poisonous heavy metals may have a major negative impact on a plant's physiological and metabolic processes, which can eventually reduce the amount of *Avena sativa* produced and cause the metals to infiltrate the food chain and harm both humans and animals. Thus, to retain and maintain plant physiological activities, soil nutrient pools, and contemporaneous oat production in an environment that is continuously degrading, metal-induced phytotoxicity issues require urgent and immediate treatment (Amanullah, Nahid, Hosen, Akther, & Kauser-Ul-Alam, 2024).

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One of the auxins with the highest physiological activity is indole acetic acid (IAA). Plant Growth-Promoting Rhizobacteria (PGPR) is one of the microorganisms that create IAA, a typical byproduct of L-tryptophan metabolism. IAA causes specific RXA and protein synthesis, increases the osmotic contents of the cell, increases the permeability of water into the cell, lowers wall pressure, and increases the synthesis of cell walls to promote cell elongation. It encourages emulation and inhibits It encourages embial activity, inhibits or postpones leaf abscission, and stimulates fruiting and flowering. Many Trp-dependent and Trp-independent processes in bacteria and plants produce the metabolite IAA from Trp. A bacterium may contain more than one route. Tryptophan-2-monooxygenase converts tryptophan to indole-3-acetamide (IAM) in the Trp-dependent pathway, while IAM-hydrolase metabolizes IAM to IAA. Horemans and Vlassak (1985) found that when *A. brasilense* was cultivated aerobically, it could create IAA without tryptophan and that the addition of NH4 resulted in the maximum amounts of auxin production. It seems to be especially significant during embryogenesis when polar development depends on precise control over low amounts of IAA. Although a large amount of Trp-to-IAA conversion also happens in such preparations, the Trp-independent pathway may contribute significantly to the newly synthesized IAA Mohite, B. (2013).

The primary goal of this study was to separate and screen naturally occurring bacteria that produce indole acetic acid from various rhizospheric soils. The second step involved filtering the IAA and testing its capacity to stimulate plant development by rhizobacteria characteristics. Additionally, optimization research was conducted to produce high levels of IAA using physicochemical parameters like temperature, pH, tryptophan supplementation, and sources of carbon and nitrogen Mohite, B. (2013).

2. Methodology

2.1 Selection of Plant Material

Brassica napus was grown under natural conditions at the Department of Botany, The Islamia University of Bahawalpur, from October 2023 to March 2024. Environmental factors such as temperature, humidity, and sunlight were suitable for plant growth.

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2.2 Soil Preparation

The soil was collected from Dera Bakha and the university's desert area, mixed in a 1:2:1 ratio (sand, clay, and manure), and filled into 5-kg polythene bags.

2.3 Moisture Maintenance

Bags were watered for two days to maintain moisture, with drainage holes added for aeration.

2.4 Seed Sowing

Certified B. napus seeds were tested for viability and sown (5 per bag, 1-inch depth) on October 10, 2023. Pots were kept in sunlight and watered regularly, with plastic sheet covers used at night for protection.

2.5 Seedling Emergence

Seedlings emerged five days post-sowing.

2.6 Soil Analysis

Soil physiochemical properties, including pH, electrical conductivity (EC), sodium adsorption ratio (SAR), and chloride content, were analyzed using standard laboratory methods.

2.7 Experimental Design

A Completely Randomized Design (CRD) was used, comprising eight treatment blocks with five replicates each. Treatments included varying Auxin (0.5mM, 2.5mM) and copper (0.5mM, 1mM, 2mM, 3mM) applications, either alone or in different combinations. Treatments were applied at 10-day intervals, starting November 6-7, 2023.2

2.8 Potted Experiment

NaCl (0.5mM–3mM) and Auxin (0.5mM, 2.5mM) treatments were applied in fixed amounts (50ml metal treatment per root; 10ml Auxin foliar spray per pot).

2.9 Preparation of Solutions

NaCl and Auxin solutions were prepared in distilled water at specified concentrations.

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2.10 Collection of Plant Material

Plants were sampled 10 days after the third treatment for morphological and anatomical analysis.

2.11 Morphological & Anatomical Parameters

- **Morphological:** Root length, shoot length, leaf count, leaf area, fresh & dry weight.
- **Anatomical:** Midrib thickness, cortex thickness, vascular bundle area, mesophyll & cortex cell area.

2.12 Slide Preparation & Microscopy

Plant samples were fixed in FAA, dehydrated, stained, and observed under a microscope for anatomical studies.

2.13 Statistical Analysis

SPSS 8.1 was used for statistical comparisons, with results presented via tables and graphs.

3. Results

3.1. Morphological parameters

3.1.1. Plant fresh weight

Figure 3.1 shows the plant fresh weight of the different treatments. There were significant differences in the plant fresh weight for different metal stress and regulator concentrations. T6 had the lowest plant fresh weight, 1.81 g, while T2 had the largest weight, 8.83 g. The plant fresh weight in control T0 was 5.31 g, but in T1 and T2, the highest and lowest amounts of Auxin were administered, respectively, and the plant fresh weight increased to 6.27 g and 8.83 g. When applying only NaCl, the plant fresh weight gradually decreased as the concentration rose from the lowest to the highest; in T3, T4, T5, and T6, the corresponding values were 5.21 g, 4.37 g, 3.91 g, and 1.81 g.

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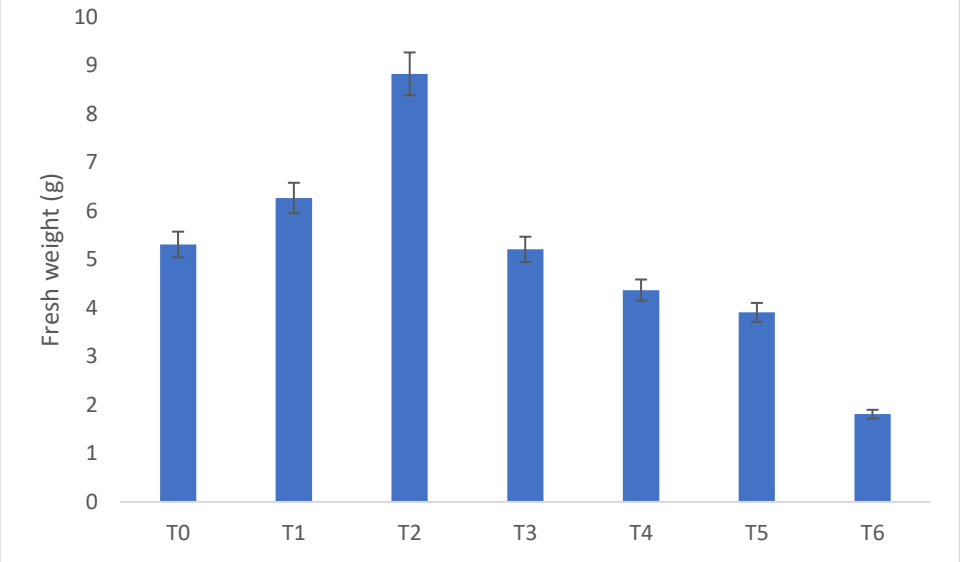

Figure 3.1: Comparison of Plant fresh weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Figure 3.2 shows that the plant in T3 that was treated with NaCl had a fresh weight of 5.21. When Auxin was administered concurrently at the highest and lowest concentrations, either before or following the NaCl treatment, the weight increased. In T15 and T19, the metal was applied one day before Auxin was applied and it showed 5.46g and 5.71g; in T23 and T27, the metal was applied one day after Auxin was applied. The corresponding weights were 5.46g and 5.71g in those cases. Auxin and metal were administered simultaneously to T7 and T11 it showed 5.37g and 5.56g respectively.

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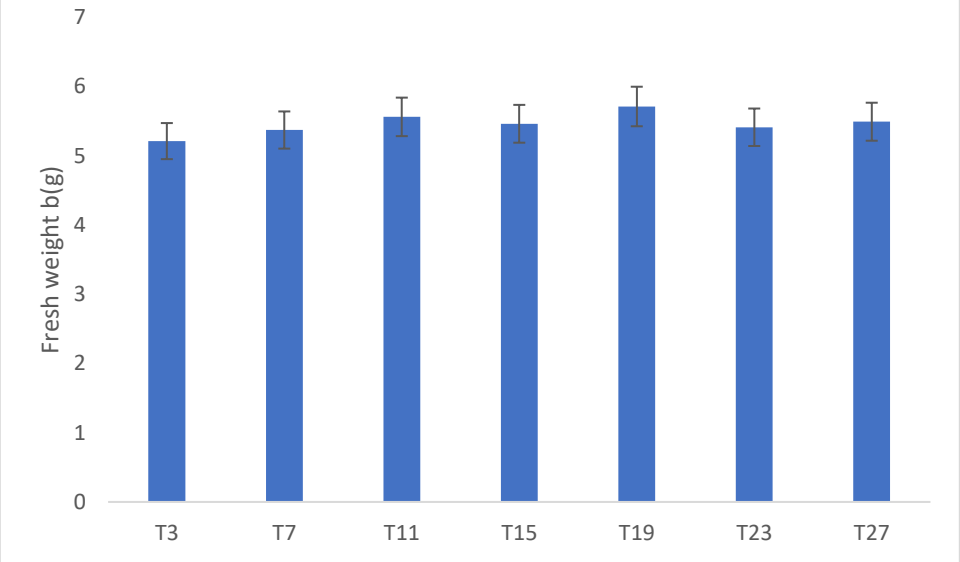

Figure 3.2: Comparison of Plant fresh weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM IAA), T11(0.5mM NaCl+ 2.5mM IAA), T15(pre 0.5mM NaCl+ post 0.5mM IAA), T19(pre 0.5mM NaCl+ post 2.5mM IAA), T23(post 0.5mM NaCl+ pre 0.5mM IAA), T27(post 0.5mM NaCl+ pre 2.5mM IAA).

In T16 and T20, where IAA and metal were applied simultaneously, there was a significant increase in weight when IAA was applied after the application of NaCl, to 4.58g and 4.77g. Plants in T4 had 4.37g, and when IAA was applied in conjunction with NaCl, either before or after the application of NaCl, the area increased to 4.51g and 4.63g in T8 and T12. T24 and T28 showed a little increase at 4.54g and 4.69g, respectively, when IAA was given one day following NaCl therapy.

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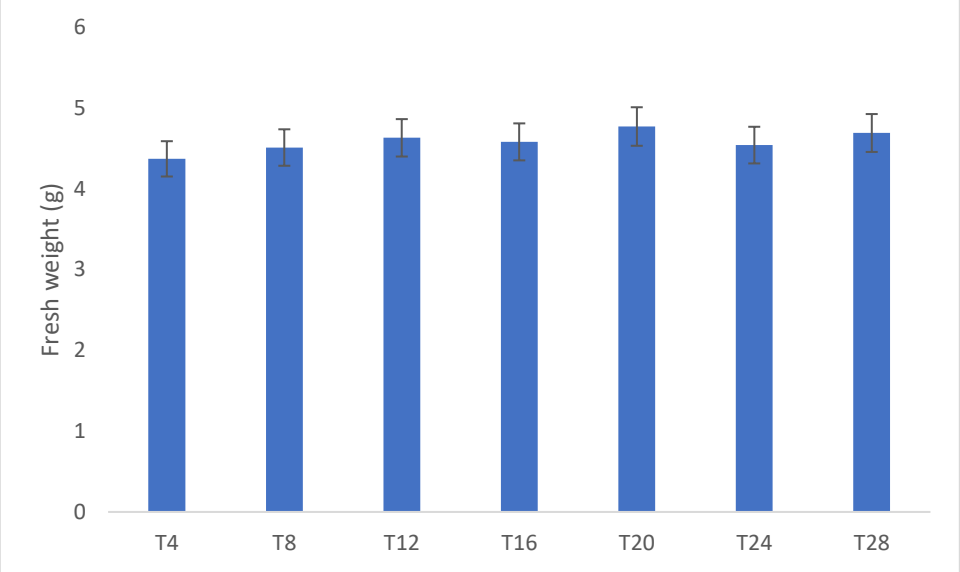

Figure 3.3: Comparison of Plant fresh weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T4(1mM NaCl), T8(1mM NaCl+ 0.5mM IAA), T12(1mM NaCl+ 2.5mM IAA), T16(pre 1mM NaCl+ post 0.5mM IAA), T20(pre 1mM NaCl+ post 2.5mM IAA), T24(post 1mM NaCl+ pre0.5mM IAA), T28(post 1mM NaCl+ pre2.5mM IAA).

Plants treated with solo NaCl in T5 had a fresh weight of 3.91g, as shown in Figure 3.3, and this weight increased when IAA was given NaCl. Nevertheless, when IAA was sprayed the day after NaCl was treated, a notable increase in root area was seen. The fresh weight of the plant was 3.17g and 3.31g in T9 and T13, 3.26g and 3.47g in T17 and T21, and 3.21g and 3.36g in T25 and T29, respectively.

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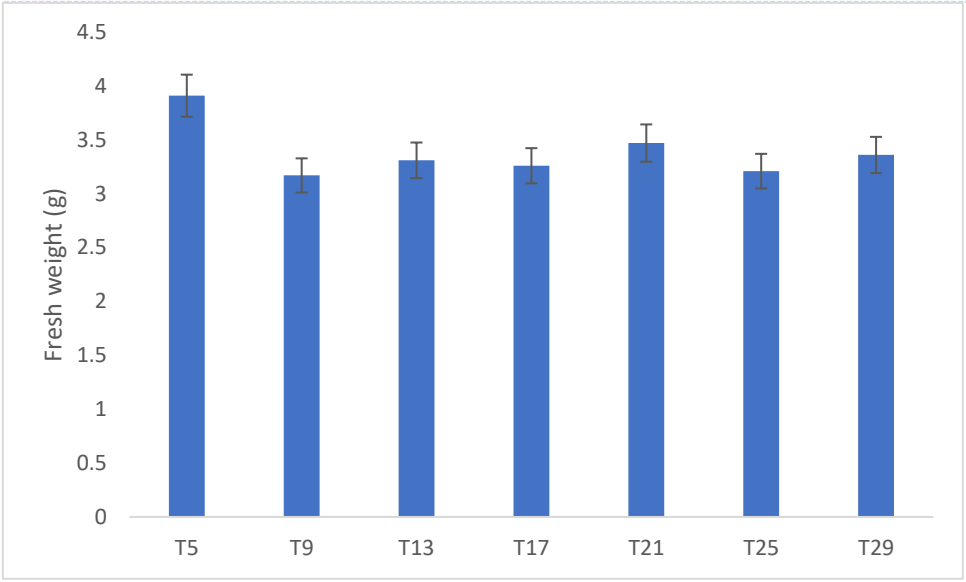

Figure 3.4: Comparison of Plant fresh weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T5(2mM NaCl), T9(2mM NaCl+ 0.5mM IAA), T13(2mM NaCl+ 2.5mM IAA), T17(pre 2mM NaCl+ post 0.5mM IAA), T21(pre 2mM NaCl+ post 2.5mM IAA), T25(post 2mM NaCl+ pre 0.5mM IAA), T29(post 2mM NaCl+ pre 2.5mM IAA).

As shown in Fig. 3.5, the minimum plant fresh weight of 1.81g in T6 was marginally increased upon the administration of both the minimum and maximum concentration of IAA. In T10 and T14, metal and IAA were applied simultaneously and weight was 1,89g and 2.03g; in T18 and T22, metal was applied one day before IAA was applied and weight was 1.96g and 2.19g respectively; and in T26 and T30, metal was applied one day after IAA was applied and the observed weight was 1.93g and 1.99 g respectively.

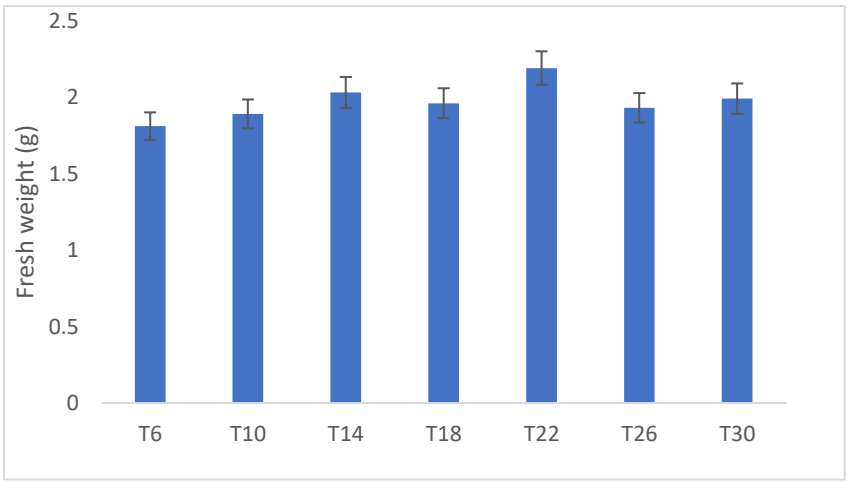

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Figure 3.5: Comparison of Plant fresh weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T6(3mM NaCl), T10(3mM NaCl+ 0.5mM IAA), T14(3mM NaCl+ 2.5mM IAA), T18(pre 3mM NaCl+ post 0.5mM IAA), T22(pre 3mM NaCl+ post 2.5mM IAA), T26(post 3mM NaCl+ pre 0.5mM IAA), T30(post 3mM NaCl+ pre 2.5mM IAA).

Plants with the highest concentration of Auxin had the maximum plant fresh weight. When compared to the single metal, treatments with lower NaCl and both regulator concentrations had maximum plant fresh weight; however, treatments where IAA was administered a day after NaCl application demonstrated a significant maximum plant fresh weight. It is demonstrated that treatments, where IAA was administered exogenously one day following low to medium amounts of NaCl, had the maximum plant fresh weight.

3.1.2. Plant Dry weight

Figure 3.6 The plant dry weight of the different treatments is shown in Figure 1. There were significant differences in the plant dry weight for different metal stress and regulator concentrations. T6 had the lowest plant dry weight, 0.06 g, while T2 had the largest weight, 0.55 g. The plant dry weight in control T0 was 0.35 g, but in T1 and T2, the highest and lowest amounts of Auxin were administered, respectively, and the plant dry weight increased to 0.41 g and 0.55 g. When applying only NaCl, the plant dry weight gradually decreased as the concentration rose from the lowest to the highest; in T3, T4, T5, and T6, the corresponding values were 0.34 g, 0.22 g, 0.11 g, and 0.06 g.

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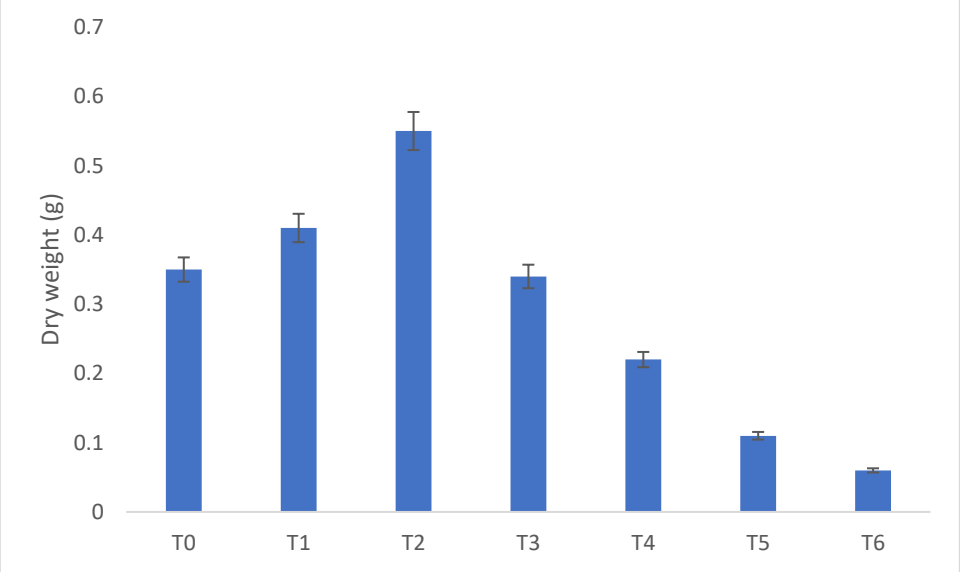

Figure 3.6: Comparison of Plant dry weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Figure 3.7 shows that the plant in T3 that was treated with NaCl had a plant dry weight of 0.34g. When Auxin was administered concurrently at the highest and lowest concentrations, either before or following the NaCl treatment, the weight increased. In T15 and T19, the metal was applied one day before Auxin was applied and it showed 0.41g and 0.49g; in T23 and T27, the metal was applied one day after Auxin was applied. The corresponding weights were 0.39g and 0.46g in those cases. Auxin and metal were administered simultaneously to T7 and T11 which showed 0.37g and 0.43g respectively.

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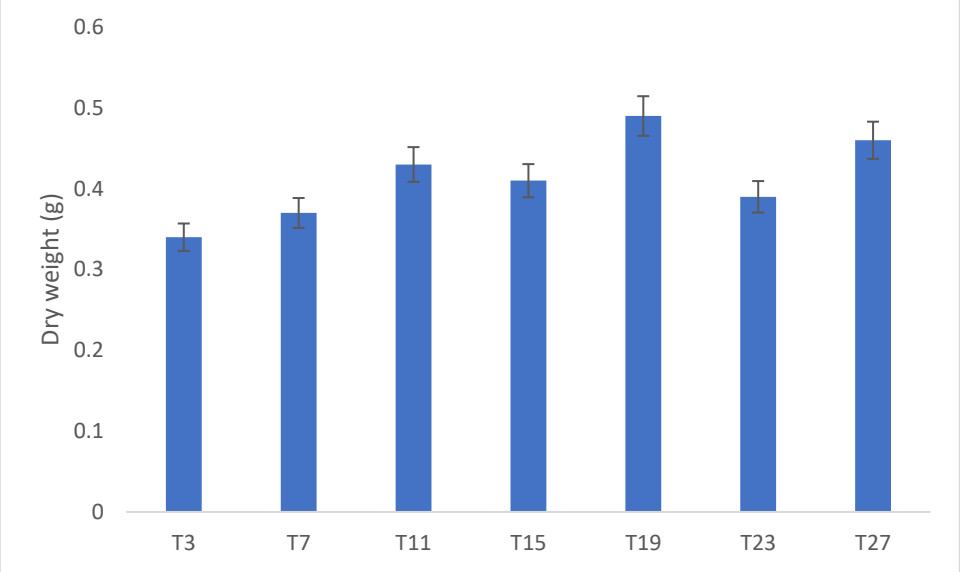

Figure 3.7: Comparison of Plant dry weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM IAA), T11(0.5mM NaCl+ 2.5mM IAA), T15(pre 0.5mM NaCl+ post 0.5mM IAA), T19(pre 0.5mM NaCl+ post 2.5mM IAA), T23(post 0.5mM NaCl+ pre 0.5mM IAA), T27(post 0.5mM NaCl+ pre 2.5mM IAA).

In T16 and T20, where IAA and metal were applied simultaneously, there was a significant increase in weight when IAA was applied after the application of NaCl, to 0.33g and 0.43g. Plants in T4 had 0.22g, and when IAA was applied in conjunction with NaCl, either before or after the application of NaCl, the area increased to 0.27g and 0.36g in T8 and T12. T24 and T28 showed a little increase at 0.30g and 0.39g, respectively, when IAA was given one day following NaCl therapy.

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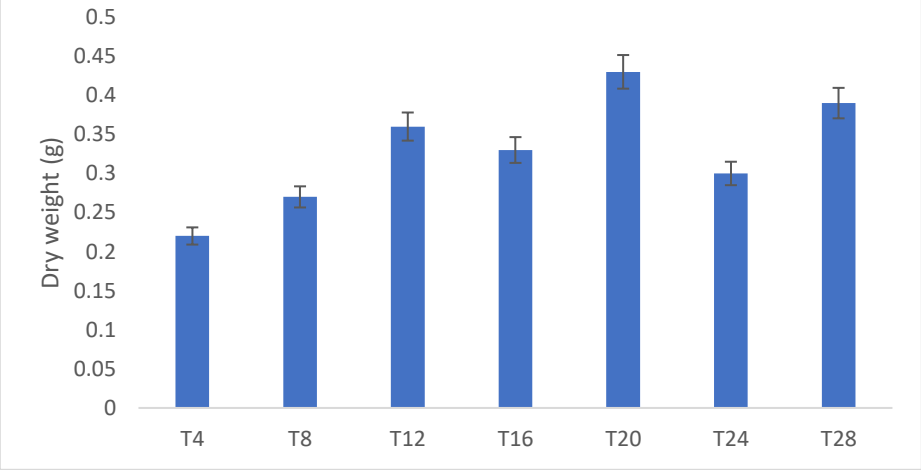

Figure 3.8: Comparison of Plant dry weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T4(1mM NaCl), T8(1mM NaCl+ 0.5mM IAA), T12(1mM NaCl+ 2.5mM IAA), T16(pre 1mM NaCl+ post 0.5mM IAA), T20(pre 1mM NaCl+ post 2.5mM IAA), T24(post 1mM NaCl+ pre 0.5mM IAA), T28(post 1mM NaCl+ pre 2.5mM IAA).

Plants treated with solo NaCl in T5 had a dry weight of 0.11g, as shown in Figure 3.9, and this weight increased when IAA was given NaCl. Nevertheless, a notable increase in plant dry weight was seen when IAA was sprayed the day after NaCl was treated. The fresh weight of the plant was 0.14g and 0.19g in T9 and T13, 0.17g and 0.26g in T17 and T21, and 0.15g and 0.22g in T25 and T29, respectively.

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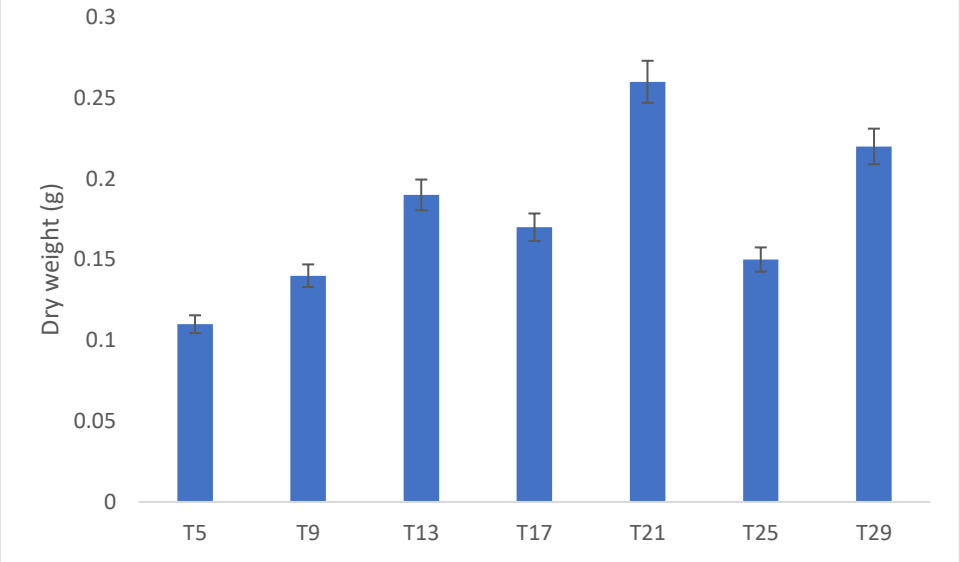

Figure 3.9: Comparison of Plant dry weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T5(2mM NaCl), T9(2mM NaCl+ 0.5mM IAA), T13(2mM NaCl+ 2.5mM IAA), T17(pre 2mM NaCl+ post 0.5mM IAA), T21(pre 2mM NaCl+ post 2.5mM IAA), T25(post 2mM NaCl+ pre 0.5mM IAA), T29(post 2mM NaCl+ pre 2.5mM IAA).

As shown in Fig. 4.10, the minimum plant dry weight of 0.06g in T6 was marginally increased upon the administration of both the minimum and maximum concentration of IAA. In T10 and T14, metal and IAA were applied simultaneously and weight was 0.08g and 0.15g; in T18 and T22, metal was applied one day before IAA was applied and weight was 0.13g and 0.20g respectively; and in T26 and T30, metal was applied one day after IAA was applied and the observed weight was 0.11g and 0.17 g respectively.

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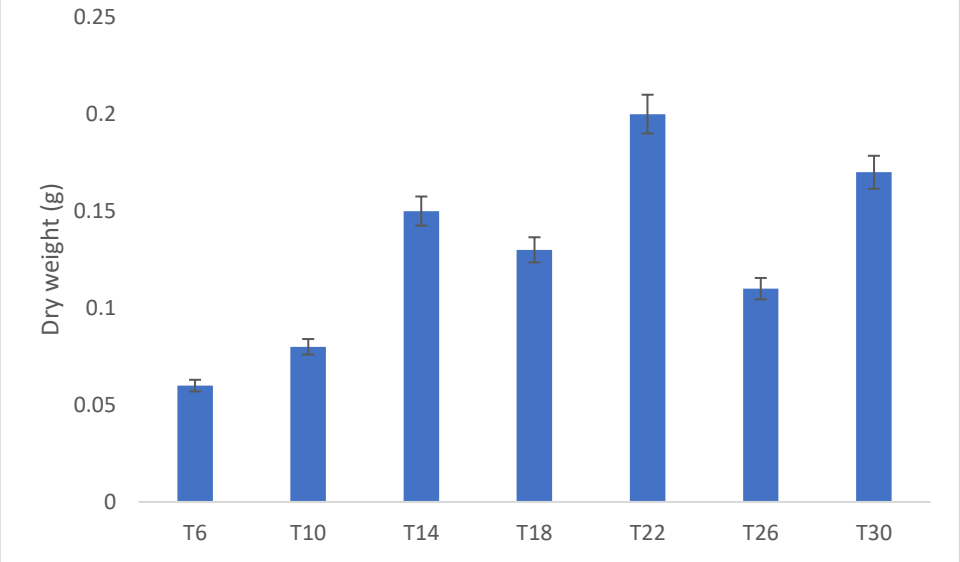

Figure 3.10: Comparison of Plant dry weight (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T6(3mM NaCl), T10(3mM NaCl+ 0.5mM IAA), T14(3mM NaCl+ 2.5mM IAA), T18(pre 3mM NaCl+ post 0.5mM IAA), T22(pre 3mM NaCl+ post 2.5mM IAA), T26(post 3mM NaCl+ pre 0.5mM IAA), T30(post 3mM NaCl+ pre 2.5mM IAA).

Plants with the highest concentration of Auxin had the maximum plant dry weight. When compared to the single metal, treatments with lower NaCl and both regulator concentrations had maximum plant fresh weight; however, treatments where IAA was administered a day after NaCl application demonstrated a significant maximum plant dry weight. It is demonstrated that treatments, where IAA was administered exogenously one day following low to medium amounts of NaCl, had the maximum plant dry weight.

3.1.3. Plant Shoot Length

Figure 3.11. the plant shoot length of the different treatments is shown in Figure 1. There were significant differences in the plant shoot length for different metal stress and regulator concentrations. T6 had the lowest plant shoot length, 12.1cm, while T2 had the largest length, 28.5cm. The plant shoot length in control T0 was 21.7cm, but in T1 and T2, the highest and lowest amounts of Auxin were administered, respectively, and the plant shoot length increased to 24.3cm and 28.5cm. When applying only NaCl, the plant shoot length

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gradually decreased as the concentration rose from the lowest to the highest; in T3, T4, T5, and T6, the corresponding values were 21.1cm, 18.3cm, 15.6cm, and 12.1cm.

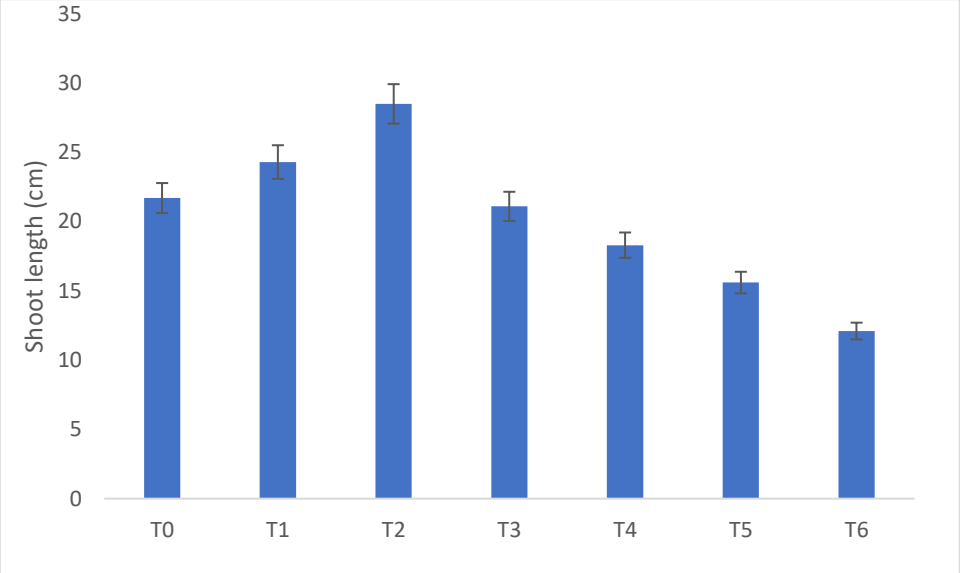

Figure 3.11: Comparison of Plant shoot length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Figure 3.1.12 showed that the plant in T3 that was treated with NaCl had a plant shoot length of 21.1cm. When Auxin was administered concurrently at the highest and lowest concentrations, either before or following the NaCl treatment, the length increased. In T15 and T19, the metal was applied one day before Auxin was applied and it showed 21.9cm and 23.6cm; in T23 and T27, the metal was applied one day after Auxin was applied. The corresponding weights were 21.6cm and 22.8cm in those cases. Auxin and metal were administered simultaneously to T7 and T11 which showed 21.4cm and 22.3cm respectively.

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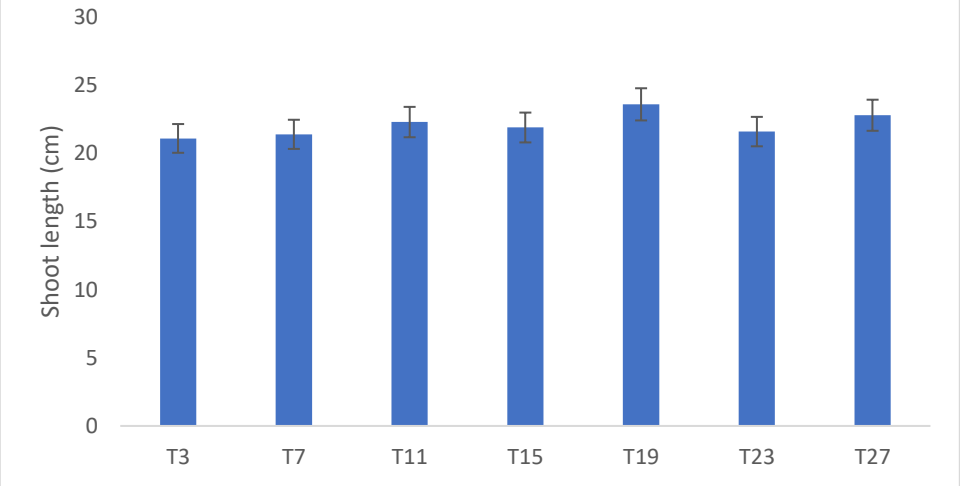

Figure 3.12: Comparison of Shoot length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM IAA), T11(0.5mM NaCl + 2.5mM IAA), T15(pre0.5mM NaCl+ post 0.5mM IAA), T19(pre 0.5mM NaCl+ post 2.5mM IAA), T23(post 0.5mM NaCl+ pre 0.5mM IAA), T27(post 0.5mM NaCl+ pre 2.5mM IAA).

In T16 and T20, where IAA and metal were applied simultaneously, there was a significant increase in length when IAA was applied after the application of NaCl, to 19.4cm and 21.1cm. Plants in T4 had 18.3cm, and when IAA was applied in conjunction with NaCl, either before or after the application of NaCl, the area increased to 18.7cm and 19.8cm in T8 and T12. T24 and T28 showed a little increase at 19.0cm and 20.3cm, respectively, when IAA was given one day following NaCl therapy.

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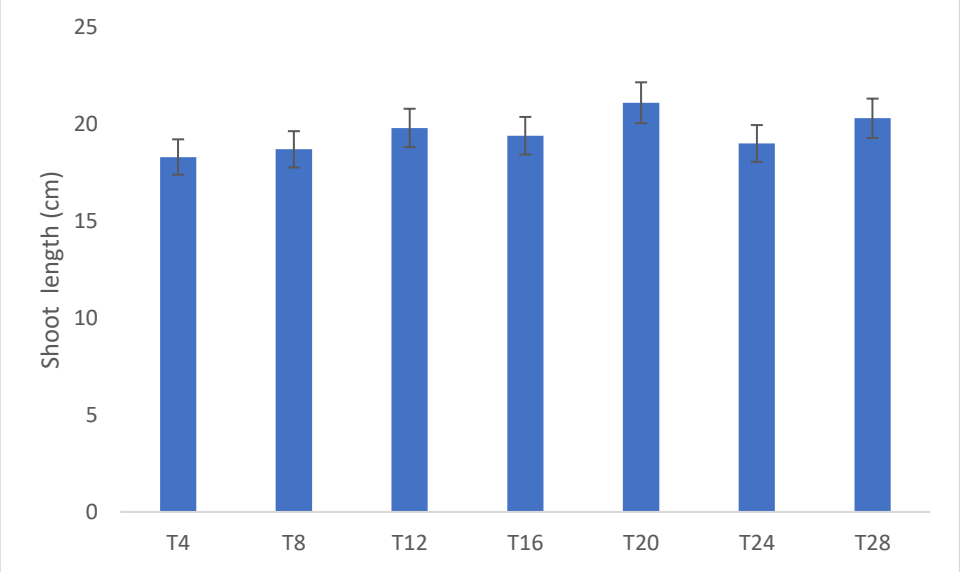

Figure 3.13: Comparison of Shoot length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T4(1mM NaCl), T8(1mM NaCl+ 0.5mM IAA), T12(1mM NaCl+ 2.5mM IAA), T16(pre 1mM NaCl+ post 0.5mM IAA), T20(pre 1mM NaCl+post 2.5mM IAA), T24(post 1mM NaCl+ pre 0.5mM IAA), T28(post 1mM NaCl+ pre 2.5mM IAA).

Plants treated with solo NaCl in T5 had a plant shoot length of 15.6cm, as shown in Figure 3.14, and this length increased when IAA was given NaCl. Nevertheless, when IAA was sprayed the day after NaCl was treated, a notable increase in plant shoot length was seen. The shoot length of the plant was 15.9cm and 16.8cm in T9 and T13, 16.5cm and 17.9cm in T17 and T21, and 16.2cm and 17.2cm in T25 and T29, respectively.

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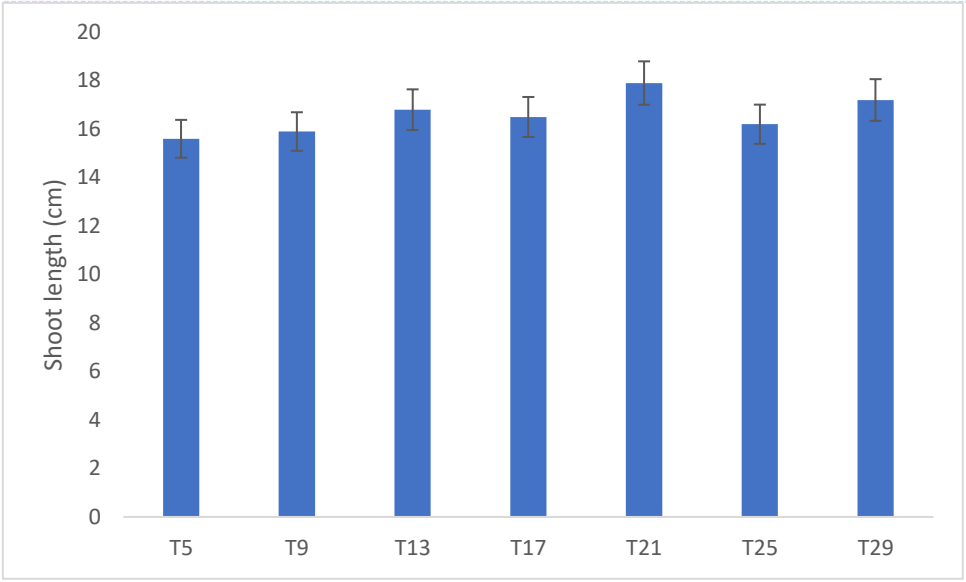

Figure 3.14: Comparison of Shoot length (g) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T5(2mM NaCl), T9(2mM NaCl+ 0.5mM IAA), T13(2mM NaCl+ 2.5mM IAA), T17(pre 2mM NaCl+ post 0.5mM IAA), T21(pre 2mM NaCl+ post 2.5mM IAA), T25(post 2mM NaCl+ pre 0.5mM IAA), T29(post 2mM NaCl+ pre 2.5mM IAA).

As shown in Fig. 3.15, the minimum plant shoot length of 12.1cm in T6 was marginally increased upon the administration of both the minimum and maximum concentration of IAA. In T10 and T14, metal and IAA were applied simultaneously and length was 12.5cm and 13.7cm; in T18 and T22, metal was applied one day before IAA was applied and weight was 13.2cm and 14.9cm respectively; and in T26 and T30, metal was applied one day after IAA was applied and the observed length was 12.9cm and 14.2cm respectively.

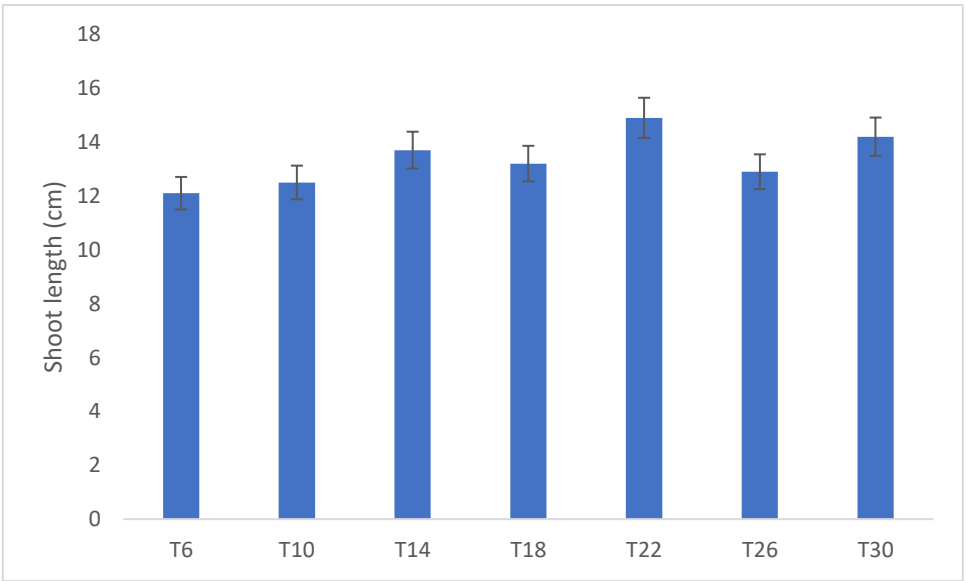

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Figure 3.15: Comparison of Shoot length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T6(3mM NaCl), T10(3mM NaCl+ 0.5mM IAA), T14(3mM NaCl+ 2.5mM IAA), T18(pre 3mM NaCl+ post 0.5mM IAA), T22(pre 3mM NaCl+ post 2.5mM IAA), T26(post 3mM NaCl+ pre 0.5mM IAA), T30(post 3mM NaCl+ pre 2.5mM IAA).

Plants with the highest concentration of Auxin had the maximum plant shoot length. When compared to the single metal, treatments with lower NaCl and both regulator concentrations had maximum plant shoot length however, treatments where IAA was administered a day after NaCl application demonstrated a significantly maximum plant shoot length. It is demonstrated that treatments, where IAA was administered exogenously one day following low to medium amounts of NaCl, had the maximum plant shoot length.

3.1.4. Plant Root Length

Figure 3.16 The plant root length of the different treatments is shown in Figure 1. There were significant differences in the plant root length for different metal stress and regulator concentrations. T6 had the lowest plant shoot length, 3.11cm, while T2 had the largest length, 8.16cm. The plant root length in control T0 was 5.66cm, but in T1 and T2, the highest and lowest amounts of Auxin were administered, respectively, and the plant root length increased to 6.78cm and 8.16cm. When applying only NaCl, the plant root length gradually decreased as the concentration rose from the lowest to the highest; in T3, T4, T5, and T6, the corresponding values were 5.6cm, 4.7cm, 3.91cm, and 3.11cm.

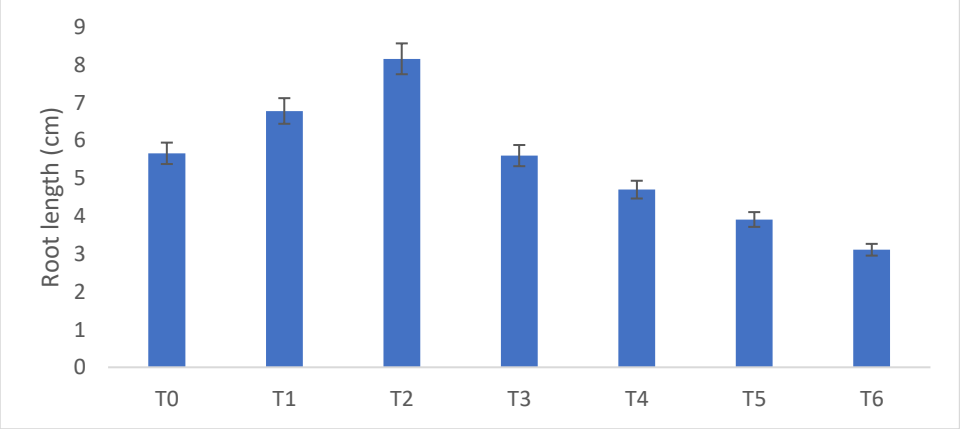

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Figure 3.16: Comparison of Root length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Figure 3.17 showed that the plant in T3 that was treated with NaCl had a plant root length of 5.6cm. When Auxin was administered concurrently at the highest and lowest concentrations, either before or following the NaCl treatment, the length increased. In T15 and T19, the metal was applied one day before Auxin was applied and it showed 5.73cm and 5.93cm; in T23 and T27, the metal was applied one day after Auxin was applied. The corresponding weights were 5.69cm and 5.83cm in those cases. Auxin and metal were administered simultaneously to T7 and T11 which showed 5.64cm and 5.77cm respectively.

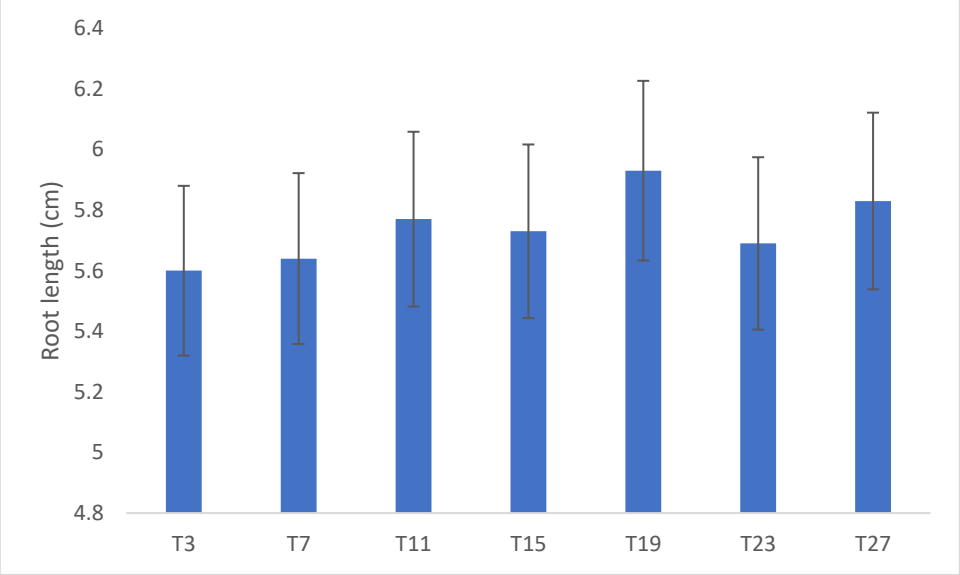

Figure 3.17: Comparison of Root length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM IAA), T11(0.5mM NaCl+ 2.5mM IAA), T15(pre 0.5mM NaCl+ post 0.5mM IAA), T19(pre 0.5mM NaCl+ post 2.5mM IAA), T23(post 0.5mM NaCl+ pre 0.5mM IAA), T27(post 0.5mM NaCl+ pre 2.5mM IAA).

In T16 and T20, where IAA and metal were applied simultaneously, there was a significant increase in length when IAA was applied after the application of NaCl, to 4.88cm and

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5.07cm. Plants in T4 had 4.7cm, and when IAA was applied in conjunction with NaCl, either before or after the application of NaCl, the area increased to 4.81cm and 4.93cm in T8 and T12. T24 and T28 showed a little increase at 4.85cm and 4.98cm, respectively, when IAA was given one day following NaCl therapy.

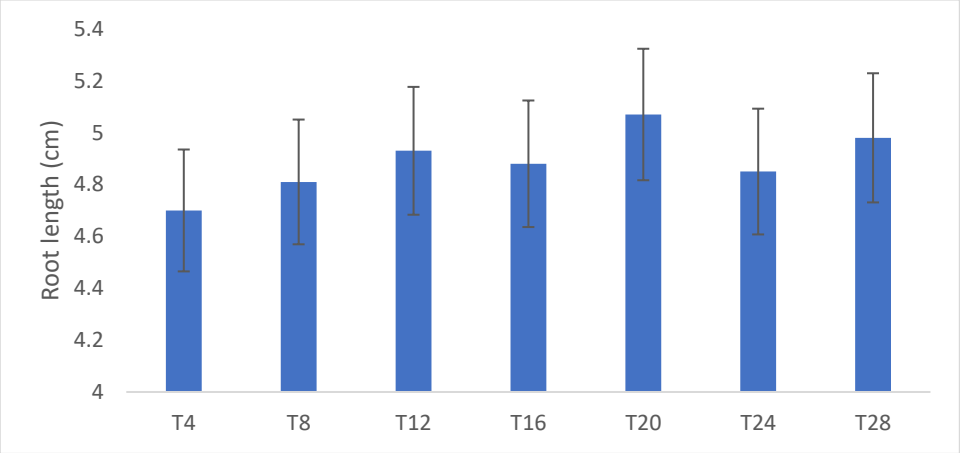

Figure 3.18: Comparison of Root length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T4(1mM NaCl), T8(1mM NaCl+ 0.5mM IAA), T12(1mM NaCl+ 2.5mM IAA), T16(pre 1mM NaCl+ post 0.5mM IAA), T20(pre 1mM NaCl+post 2.5mM IAA), T24(post 1mM NaCl+ pre 0.5mM IAA), T28(post 1mM NaCl+ pre 2.5mM IAA).

Plants treated with solo NaCl in T5 had a plant shoot length of 3.91cm, as shown in Figure 3.19, and this length increased when IAA was given NaCl. Nevertheless, when IAA was sprayed the day after NaCl was treated, a notable increase in plant root length was seen. The shoot length of the plant was 3.99cm and 4.12cm in T9 and T13, 4.08cm and 4.23cm in T17 and T21, and 4.03cm and 4.18cm in T25 and T29, respectively.

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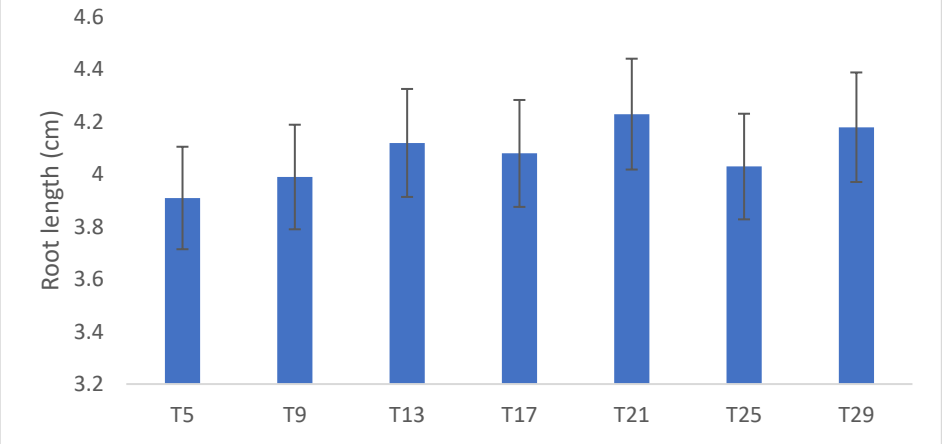

Figure 3.19: Comparison of Root length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T5(2mM NaCl), T9(2mM NaCl+ 0.5mM IAA), T13(2mM NaCl+ 2.5mM IAA), T17(pre 2mM NaCl+ post 0.5mM IAA), T21(pre 2mM NaCl+ post 2.5mM IAA), T25(post 2mM NaCl+ pre 0.5mM IAA), T29(post 2mM NaCl+ pre 2.5mM IAA).

As shown in Fig. 3.20, the minimum plant root length of 3.11cm in T6 was marginally increased upon the administration of both the minimum and maximum concentration of IAA. In T10 and T14, metal and IAA were applied simultaneously and length was 3.14cm and 3.26cm; in T18 and T22, metal was applied one day before IAA was applied and weight was 3.10cm and 3.34cm respectively; and in T26 and T30, metal was applied one day after IAA was applied and the observed length was 3.15cm and 3.26cm respectively.

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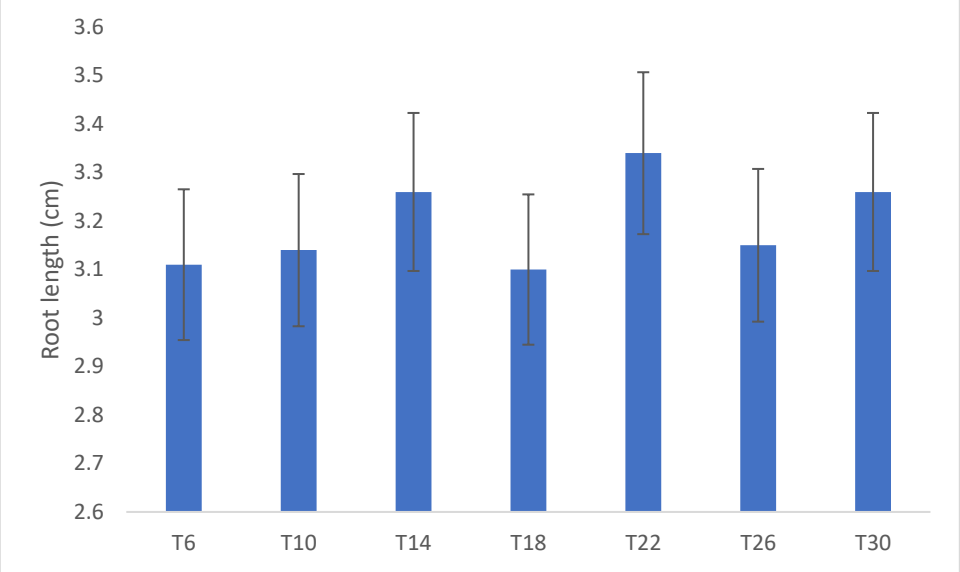

Figure 3.20: Comparison of Root length (cm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T6(3mM NaCl), T10(3mM NaCl+ 0.5mM IAA), T14(3mM NaCl+ 2.5mM IAA), T18(pre 3mM NaCl+ post 0.5mM IAA), T22(pre 3mM NaCl+ post 2.5mM IAA), T26(post 3mM NaCl+ pre 0.5mM IAA), T30(post 3mM NaCl+ pre 2.5mM IAA).

Plants with the highest concentration of Auxin had the maximum plant root length. When compared to the single metal, treatments with lower NaCl and both regulator concentrations had maximum plant root length however, treatments where IAA was administered a day after NaCl application demonstrated a significantly maximum plant root length. It is demonstrated that treatments, where IAA was administered exogenously one day following low to medium amounts of NaCl, had the maximum plant root length.

3.2. Anatomical parameters

Comparison of anatomical parameters of the leaf between different levels of Sodium Chloride, Auxin, and Sodium Chloride + Auxin when applied at different time

3.2.1. Midrib Thickness(μm²)

The differences in midrib thickness between the different treatments are shown in Figure 3.21. Significant variations were observed for different concentrations of regulators and

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mental stress. T2 exhibited the biggest midrib thickness, measuring 3.9µm, while T6 had the lowest, measuring 2.03µm. In T0, the midrib thickness was measured at 3.4µm. However, after the lowest and maximum amounts of Auxin were given, in T1 and T2, the midrib thickness increased to 3.7µm and 3.9µm, respectively. The midrib thickness reduced progressively when only NaCl was applied; in T3, T4, T5, and T6, the corresponding values were 2.4µm, 2.2µm, 2.1µm, and 2.03µm. The midrib thickness decreased from the lowest to the maximum concentration.

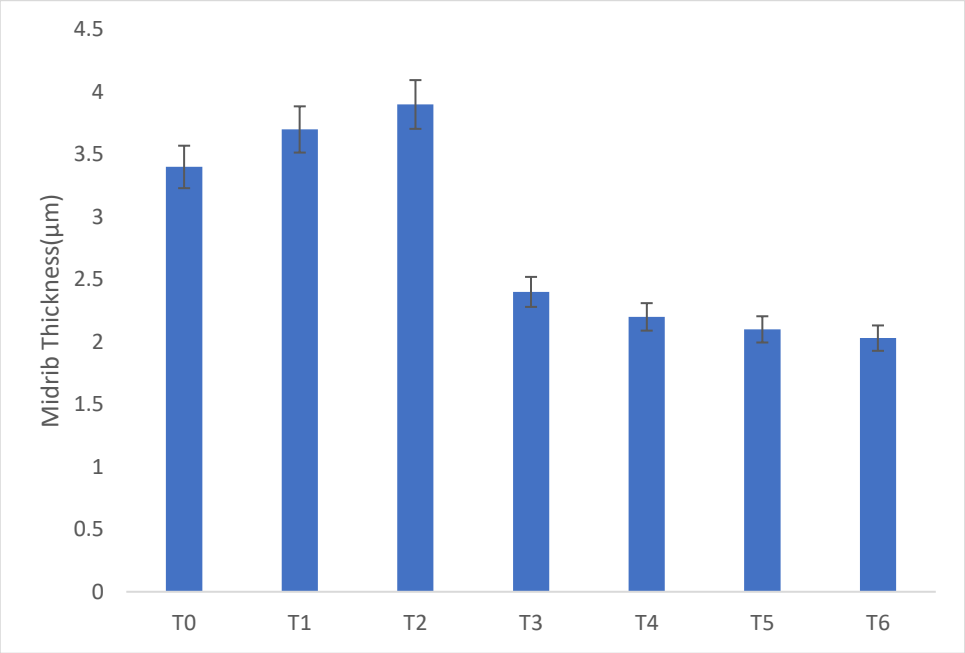

Figure 3.21: Comparison of Midrib Thickness(µm) between different levels of NaCl, IAA, and NaCl + IAA when applied at different times.

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

The plant in T3 that was treated with NaCl had a midrib thickness of 2.4µm, as shown in Figure 3.2.2. When Auxin was administered concurrently at the greatest and lowest doses, either before or following the CrCl2 treatment, the thickness of the midribs increased. In T15 and T19, the metal was applied one day before Auxin was applied; in T23 and T27, the metal was applied one day after Auxin was applied. The corresponding values were 4.4µm

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and 1.1µm in those cases. Auxin and metal were administered simultaneously to T7 and T11.

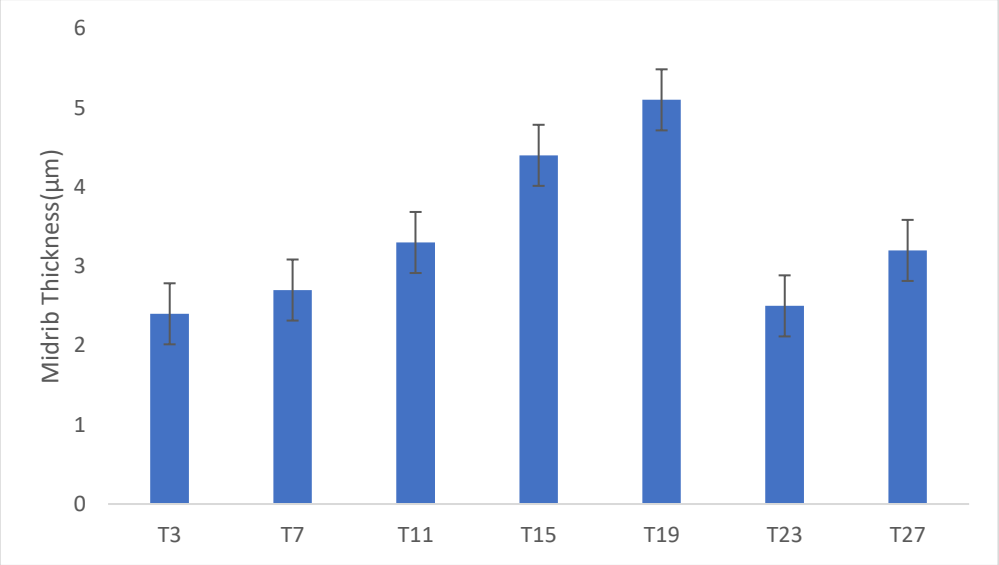

Figure 3.22: Comparison of Midrib Thickness(µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + IAA when applied at different times.

T3(0.5Mm NaCl), T7(0.5mM NaCl + 0.5mM IAA), T11(0.5mM NaCl + 2.5mM IAA), T15(pre-0.5mM NaCl + post 0.5mM IAA), T19(pre-0.5mM NaCl + post 2.5mM IAA), T23(post 0.5mM NaCl + pre-0.5mM IAA), T27(post 0.5mM NaCl + pre-2.5mM IAA).

The plants in T4 exhibited a midrib thickness of 2.2µm. In T8 and T12, the midrib thickness increased to 2.6µm and 3.2µm when IAA was applied in conjunction with NaCl, either before or following the application of NaCl. In T16 and T20, where IAA and metal were applied concurrently, there was a notable increase in midrib thickness when IAA was applied following the application of NaCl, to 4.2µm and 4.6µm in T20. When IAA was given one day before NaCl therapy, a little increase was observed in T24 and T28 at 2.5µm and 3.1µm, respectively.

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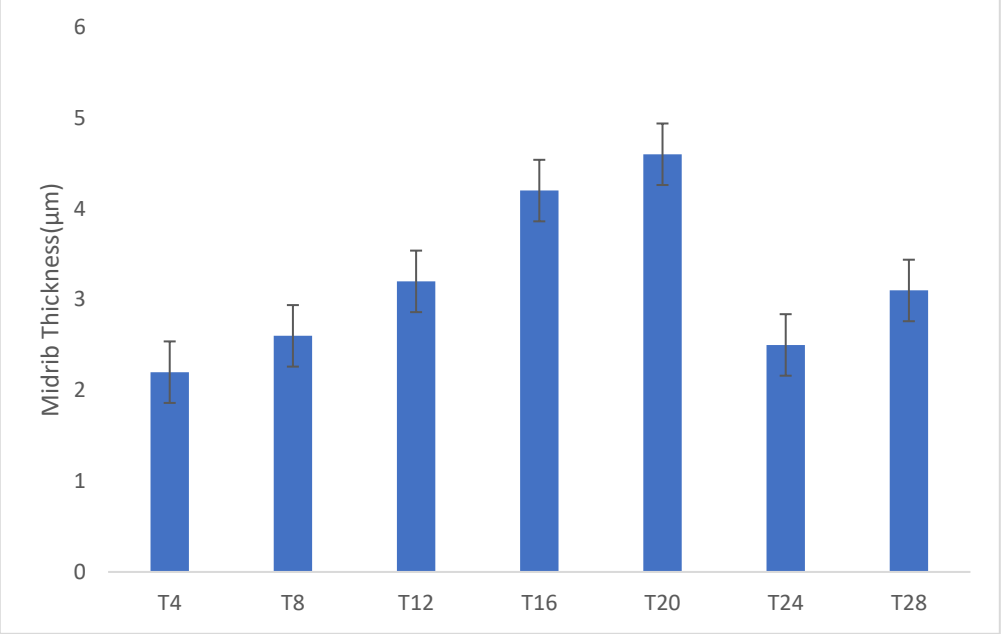

Figure 3.23: Comparison of Midrib Thickness(µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different times.

T4(1mM NaCl), T8(1mM NaCl + 0.5mM IAA), T12(pre1mM NaCl + post 2.5mM IAA), T16(pre 1mM NaCl + post 0.5mM IAA), T20(post 1mM NaCl + pre-2.5mM IAA), T24(post 1mM NaCl + pre-0.5mM IAA), T28(1mM NaCl + 2.5mM IAA).

The plants treated with solo NaCl in T5 had a midrib thickness of 2.1µm, as shown in Figure 4.24. When IAA was given with NaCl, this midrib thickness increased. However, when IAA was treated the day after NaCl was applied, there was a noticeable increase in midrib thickness. The midrib thickness was found to be 2.8 µm and 3.4 µm in T9 and T13, 3.8 µm and 4.8 µm in T17 and T21, and 2.6 µm and 3.3 µm in T25 and T29, respectively.

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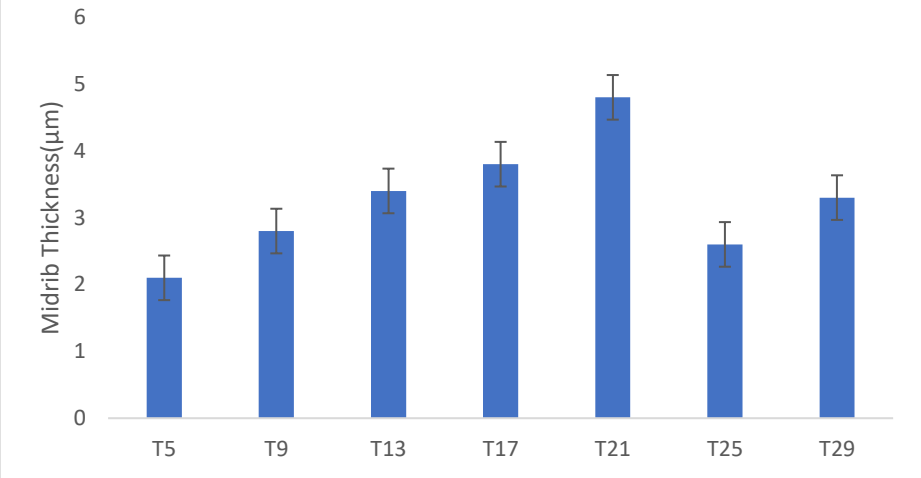

Figure 3.24: Comparison of Midrib Thickness(µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different times.

T5(2mM), T9(2mM NaCl + 0.5mM IAA), T13(2mM NaCl + 2.5mM IAA), T17(pre 2mM NaCl + post 0.5mM IAA), T21(pre 2mM NaCl + post 2.5mM IAA), T25(post 2mM NaCl + pre-0.5mM IAA), T29(post 2mM NaCl + pre-2.5mM IAA).

As shown in Fig. 3.2.5, the administration of both the minimum and maximum concentration of IAA caused T6 to have a slightly elevated minimum midrib thickness of 2.03µm. Midrib thickness measurements were as follows: 4.06µm and 4.9µm in T18 and T22, where metal was applied one day before IAA application; 2.7µm and 2.5µm in T26 and T30, where metal was applied one day after IAA application; and 2.9µm and 3.1µm in T10 and T14, where metal and IAA were applied simultaneously.

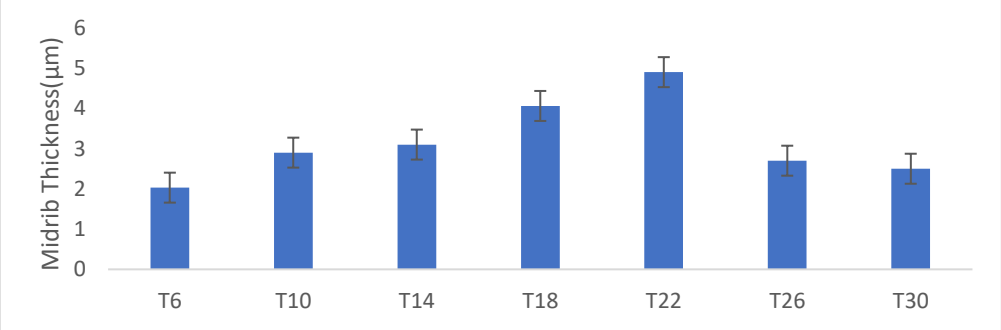

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Figure 3.25: Comparison of Midrib Thickness(µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different times.

T6(3mM NaCl), T10(3mM NaCl + 0.5mM IAA), T14(3mM NaCl + 2.5mM IAA), T18(pre 3mM NaCl + post 0.5mM IAA), T22(pre 3mM NaCl + post 2.5mM IAA), T26(post 3mM NaCl + pre-0.5mM IAA), T30(post 3mM NaCl + pre-2.5mM IAA).

The thickest midribs were found in plants that had the highest Auxin content. Treatments with lower concentrations of NaCl and both regulators showed thicker midribs compared to the single metal; treatments where IAA was added a day after NaCl application showed thicker midribs. Treatments with exogenous IAA given one day after low to medium levels of NaCl have been shown to have the highest midrib thickness.

3.2.2. Vascular bundle area (µm^2)

The difference in vascular bundle area of different treatments is shown in Fig. 4.26. Significant variations in the vascular bundle area were observed under different concentrations of metal stress and regulators. Minimum vascular bundle area was observed in T6 which was 2.97µm^2 and maximum vascular bundle area was observed in T2 which was 9.03µm^2. The vascular bundle area was 7.23µm^2 in control T0 and was increased to 7.87µm^2 and 9.03µm^2 in T1 and T2 in which minimum and maximum concentrations of Auxin were applied respectively. When sole NaCl was applied the vascular bundle area showed a gradual decrease as the concentration increased from minimum to maximum it was 7.31µm^2, 5.87µm^2, 4.23µm^2 and 2.97µm^2 in T_3, T_4, T_5 and T_6 respectively.

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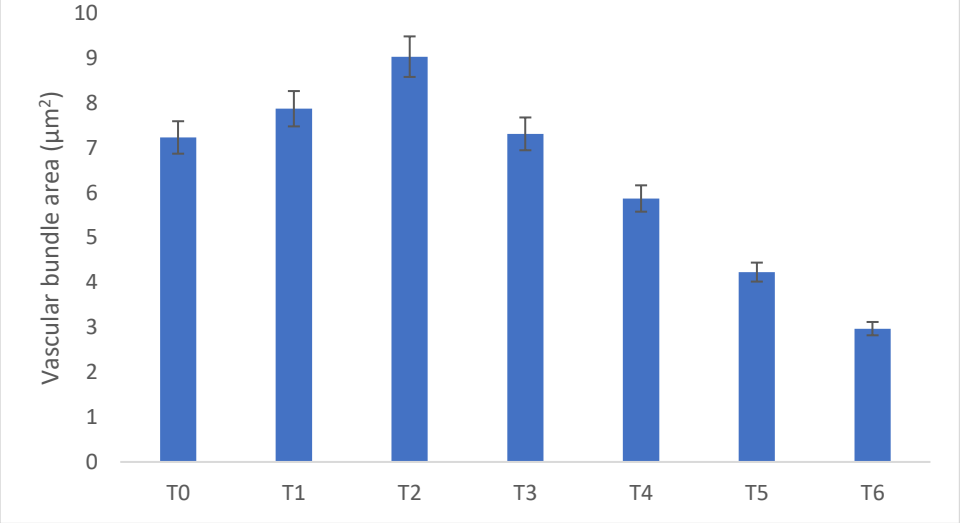

Figure 3.26: Comparison of vascular bundle area (µm^2) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Fig. 3.2.7 shows that the plant under treatment of NaCl in T_3 showed a 7.31µm^2 vascular bundle area. The increase in the area occurred when treated with minimum and maximum concentrations of Auxin at the same time, pre or post of the NaCl application. It was 7.79µm^2 and 8.22µm^2 in T_7 and T_{11} in which both Auxin and metal were applied at the same time, 7.99µm^2 and 8.37µm^2 in T_{15} and T_{19} in which metal was applied a day before the application of Auxin and 7.93µm^2 and 8.22µm^2 in T_{23} and T_{27} in which metal was applied a day after the application of Auxin respectively.

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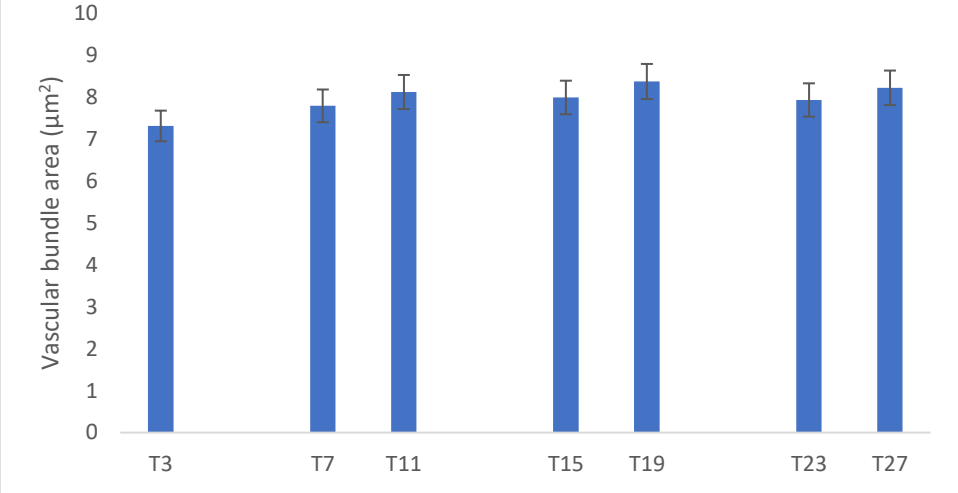

Figure 3.27: Comparison of vascular bundle area (µm^2) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM NaCl), T11(0.5mM NaCl + 2.5mM IAA), T15(pre0.5mM NaCl + post 0.5mM IAA), T19(pre0.5mM NaCl + post 2.5mM IAA), T23(post 0.5mM NaCl + pre0.5mM IAA), T27(post 0.5mM NaCl + pre2.5mM IAA).

As shown in Plants in T_4 had a 5.87µm^2 area, when IAA was applied at the same time with NaCl, the pre-or post of application of NaCl showed an increase in the area it was 6.05µm^2 and 6.45µm^2 in T_8 and $T_{12,}$ in which IAA and metal were applied at Same time, a huge increase in area was observed when IAA was applied after the application of NaCl and it was 6.13µm^2 and 6.97µm^2 in T_{16} and T_{20}. While a slight increase was observed when IAA was applied a day after the application of NaCl it was 6.09µm^2 and 6.55µm^2 in T_{24} and T_{28} respectively.

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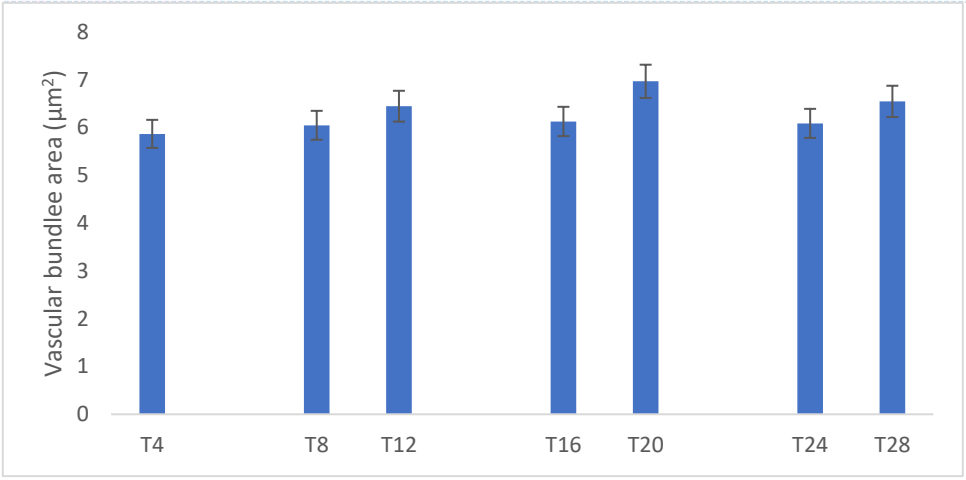

Figure 3.28: Comparison of vascular bundle area (µm^2) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T4(1mM NaCl), T8(1mM NaCl + 0.5mM NaCl), T12(pre1mM NaCl + post 2.5mM IAA), T16(pre 1mM NaCl + post 0.5mM IAA), T20(post 1mM NaCl + pre2.5mM IAA), T24(post 1mM NaCl + pr 0.5mM IAA), T28(1mM NaCl + 2.5mM IAA).

Fig 3.2.9. shows that when plants were treated with sole NaCl in T_5 the vascular bundle area was 4.23µm^2 and an increase in area occurred when IAA was applied with NaCl, but when IAA was applied a day after the application of NaCl notable increase in vascular bundle area was observed. It was 4.46µm^2 and 4.87µm^2 in T_9 and T_{13}, 4.66µm^2 and 5.12µm^2 in T_{17} and T_{21}, and 4.56µm^2 and 5.0µm^2 in T_{25} and T_{29} respectively.

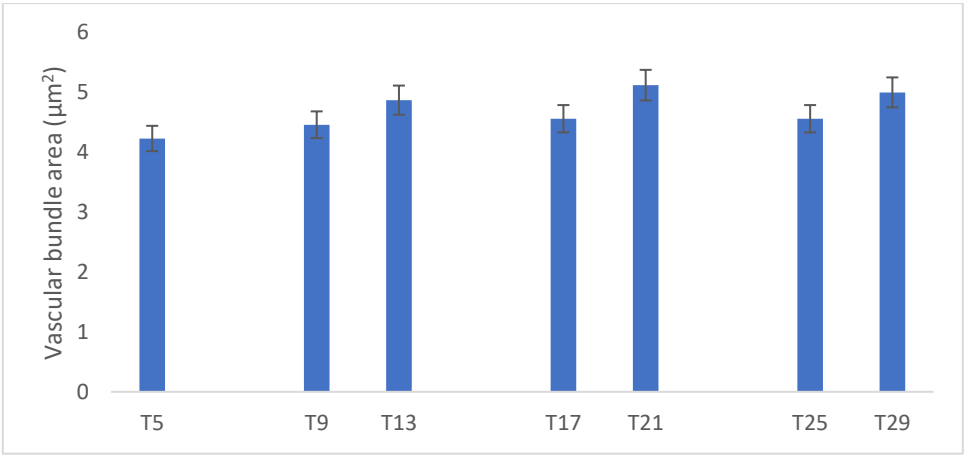

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Figure 3.29: Comparison of vascular bundle area (µm^2) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time T5(2mM NaCl), T9(2mM NaCl + 0.5mM NaCl), T13(2mM NaCl + 2.5mM IAA), T17(pre 2mM NaCl + post 0.5mM IAA), T21(pre 2mM NaCl + post 2.5mM IAA), T25(post 2mM NaCl + pre0.5mM IAA), T29(post 2mM NaCl + pre2.5mM IAA).

Figure 3.3.0 shows that in T_6 minimum vascular bundle area was observed which was 2.97µm^2 and it was slightly elevated with the application of both minimum and maximum concentration of IAA. The observed area was 3.13µm^2 and 3.37µm^2 in T_{10} and T_{14} in which both IAA and metal were applied at the same time, 3.31µm^2 and 3.77µm^2 in T_{18} and T_{22} in which metal was applied a day before the application of IAA while it was 3.21µm^2 and 3.45µm^2 in T_{26} and T_{30} in which metal was applied a day after the application of IAA respectively.

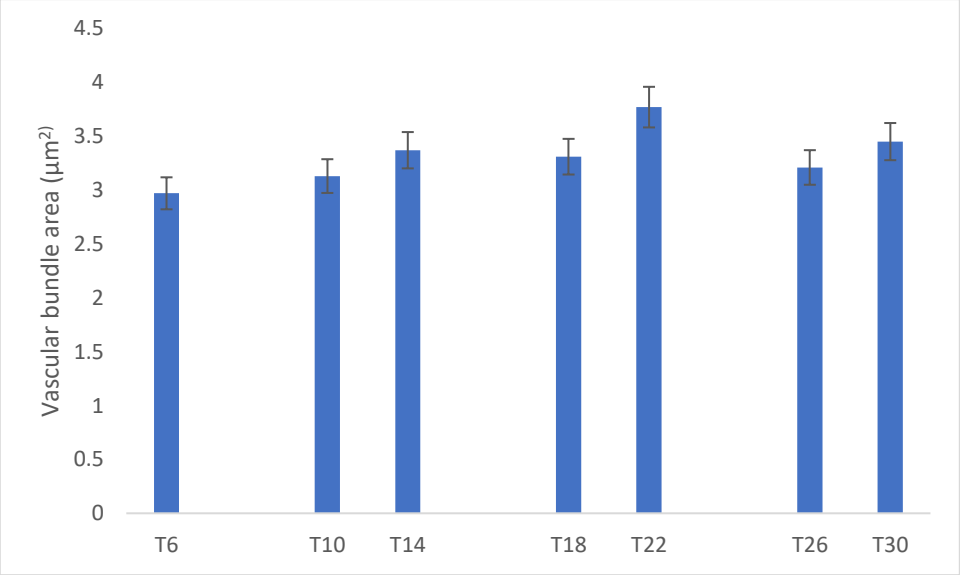

Figure 3.30: Comparison of vascular bundle area (µm^2) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time **T6(3mM NaCl), T10(3mM NaCl + 0.5mM NaCl), T14(3mM NaCl + 2.5mM IAA), T18(pre 3mM NaCl + post 0.5mM IAA), T22(pre 3mM NaCl + post 2.5mM IAA), T26(post 3mM NaCl + pre0.5mM IAA), T30(post 3mM NaCl + pre2.5mM IAA).**

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Plants to which maximum Auxin was applied showed maximum vascular bundle area. Treatments with less NaCl and both concentrations of regulator showed enhanced vascular bundle area as compared to the sole metal, but the treatments in which IAA was applied a day after the application of NaCl showed a notable increase in vascular bundle area. It is shown that enhanced vascular bundle area was observed in treatments in which IAA was applied exogenously a day after low to medium concentrations of NaCl.

3.2.3. Middle lamella

It is shown in the figure 3.3.1 that leaf middle lamella was 0.8 in control T0 and was increased to 0.9 and 1.13 in T1 and T2 respectively. When NaCl was applied the showed gradual decrease as the concentration increased from minimum to maximum it was 0.7, 0.61g, 0.44 and 0.34 in T_3, T_4, T_5 and T_6 respectively.

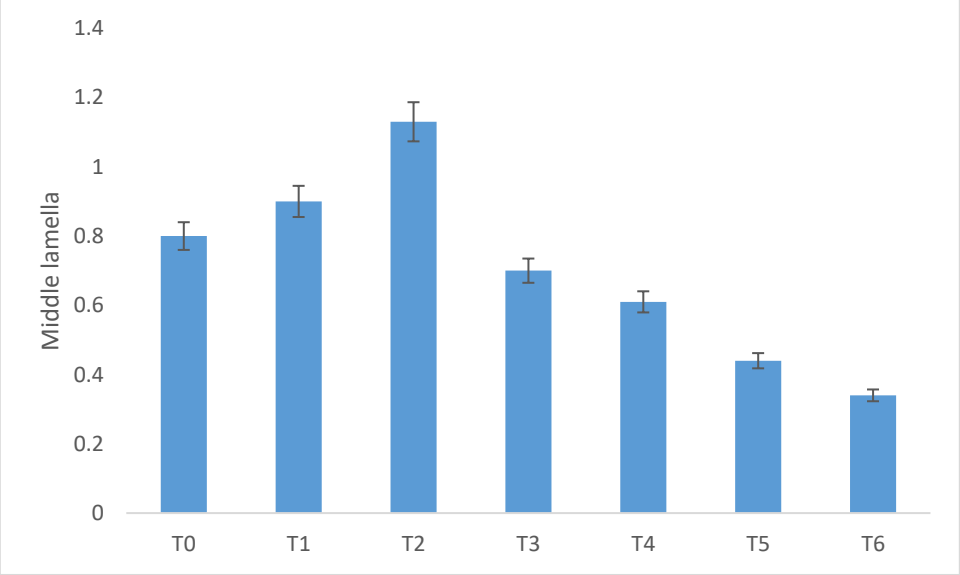

Figure 3.31: Comparison of middle lamella between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Maximum middle lamella was observed under T_{19} which was 1.75 while T_6 showed minimum thickness which was 0.34. The plant under treatment of NaCl in T_3 showed 0.7 of middle lamella thickness. The increase in thickness occurred when treated with minimum

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and maximum concentrations of Auxin at Same time, pre or post of the NaCl application. It was 1.05 and 1.3 in T_7 and T_{11}, 1.21 and 1.75 in T_{15} and T_{19} and 1.10 and 1.2 in T_{23} and T_{27} respectively.

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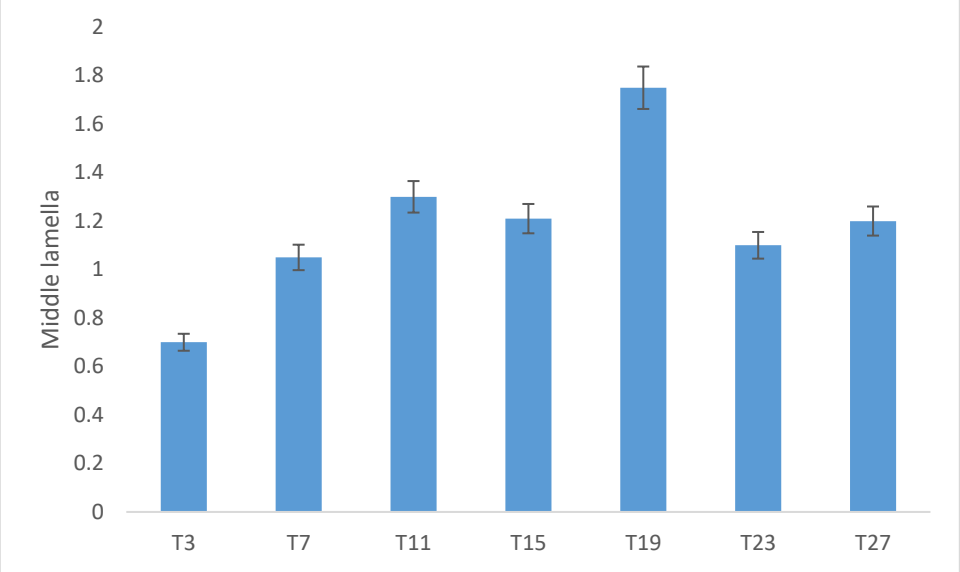

Figure 3.32: Comparison of middle lamella between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM IAA), T11(0.5mM NaCl + 2.5mM IAA), T15(pre 0.5mM NaCl + post 0.5mM IAA), T19(pre 0.5mM NaCl + post 2.5mM IAA), T23(post 0.5mM NaCl + pre 0.5mM IAA), T27(post 0.5mM NaCl + pre 2.5mM IAA).

Plants in T_4 had 0.61 when IAA was applied at the same time with NaCl, pre- or post of application of NaCl it showed an increase in thickness was 0.84 and 1.15 in T_8 and T_{12}, a huge increase in thickness was observed when IAA was applied after the application of NaCl and it was 1.01 and 1.54 in T_{16} and T_{20}. While a slight increase was observed when IAA was applied a day after the application of NaCl it was 0.93 and 1.01 in T_{24} and T_{28} respectively.

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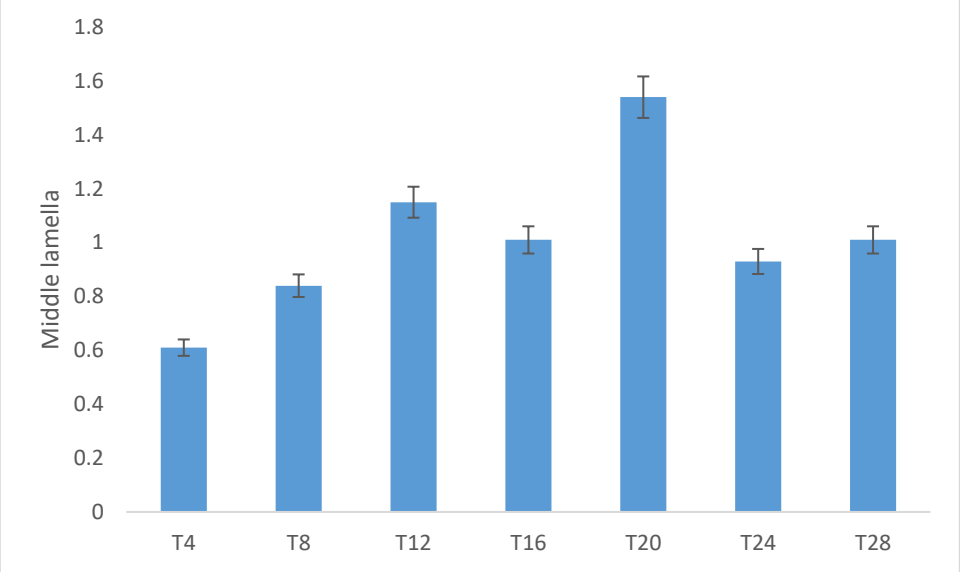

Figure 3.33: Comparison of middle lamella between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T4(1mM NaCl), T8(1mM NaCl + 0.5mM IAA), T12(1mM NaCl + 2.5mM IAA), T16(pre 1mM NaCl + post 0.5mM IAA), T20(pre 1mM NaCl +post 2.5mM IAA), T24(post 1mM NaCl + pre 0.5mM IAA), T28(post 1mM NaCl + pre 2.5mM IAA).

When plants were treated with NaCl in T_5 the middle lamella thickness was 0.44 and increase in weight occurred when IAA was applied, but when IAA was applied a day after the application of NaCl notable increase in middle lamella thickness was observed. Middle lamella thickness t was 0.5 and 0.93 in T_9 and T_{13}, 0.7 and 1.26 in T_{17} and T_{21} and 0.60 and 0.80 in T_{25} and T_{29} respectively.

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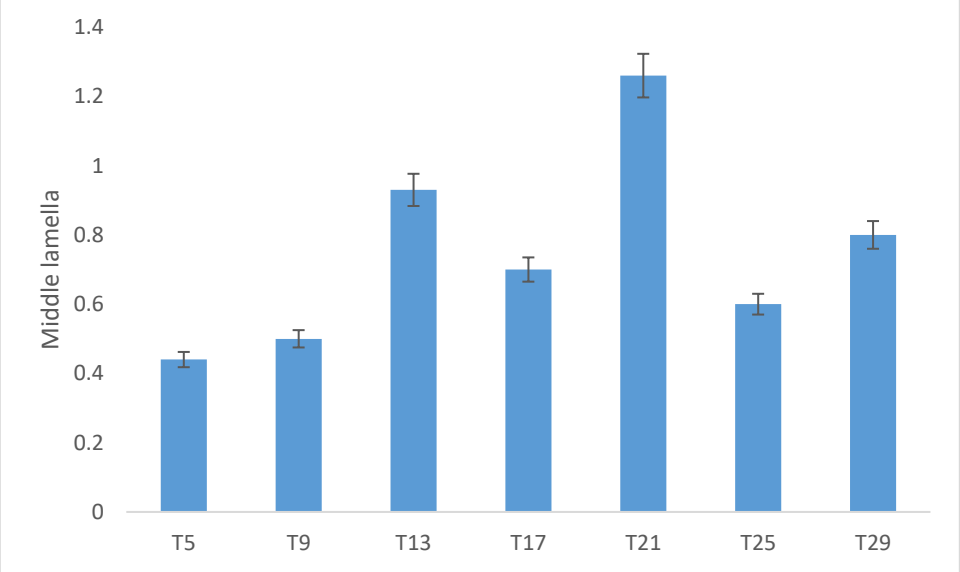

Figure 3.34: Comparison of middle lamella between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T5(2mM NaCl), T9(2mM NaCl + 0.5mM IAA), T13(2mM NaCl + 2.5mM IAA), T17(pre 2mM NaCl + post 0.5mM IAA), T21(pre 2mM NaCl + post 2.5mM IAA), T25(post 2mM NaCl + pre 0.5mM IAA), T29(post 2mM NaCl + pre 2.5mM IAA).

In T_6 middle lamella thickness was 0.34 which was slightly elevated with the application of IAA. Observed thickness was 0.45 and 0.78 in T_{10} and T_{14}, 0.60 and 0.9 in T_{18} and T_{22} while it was 0.40 and 0.62 in T_{26} and T_{30} respectively. T_1 and T_2 weight was 0.9 and 1.13 which was higher than thickness observed in T_0 which showed 0.8 middle lamella thickness.

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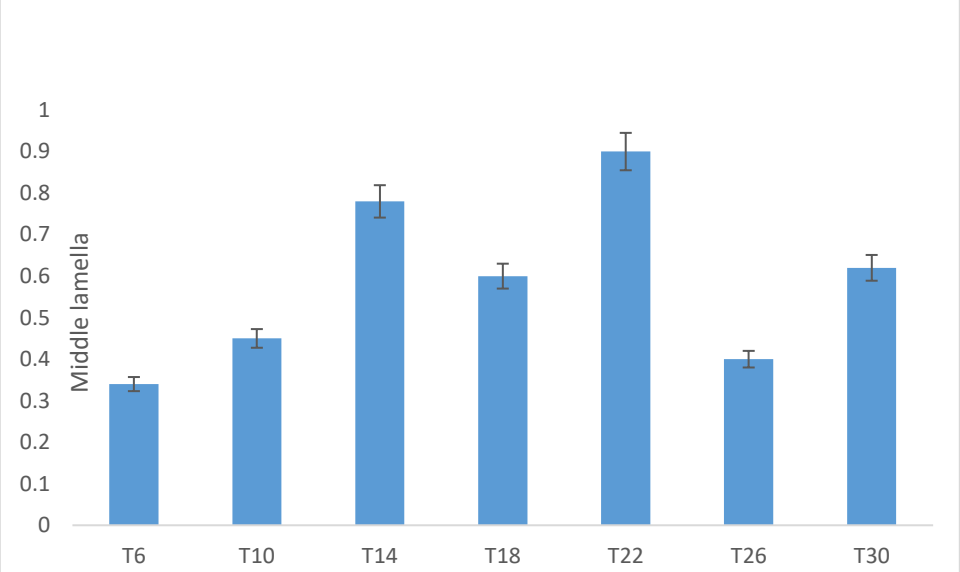

Figure 3.35: Comparison of middle lamella between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T6(3mM NaCl), T10(3mM NaCl + 0.5mM IAA), T14(3mM NaCl + 2.5mM IAA), T18(pre 3mM NaCl + post 0.5mM IAA), T22(pre 3mM NaCl + post 2.5mM IAA), T26(post 3mM NaCl + pre 0.5mM IAA), T30(post 3mM NaCl + pre 2.5mM IAA).

Epidermis thickness (µm)

The difference in epidermis thickness of different treatments is shown in Fig. 3.36. Significant variations in the epidermis thickness were observed under different concentrations of metal stress and regulator. Minimum thickness was observed in T6 which was 0.36µm and maximum epidermis thickness was observed in T2 which was 0.83µm. Epidermis thickness was 0.66µm in control T0 and was increased to 0.72µm and 0.83µm in T1 and T2 in which minimum and maximum concentrations of Auxin were applied respectively. When sole NaCl was applied the epidermis thickness showed a gradual decrease as the concentration increased from minimum to maximum it was 0.63µm, 0.54µm, 0.47µm and 0.36µm in T_3, T_4, T_5, and T_6 respectively.

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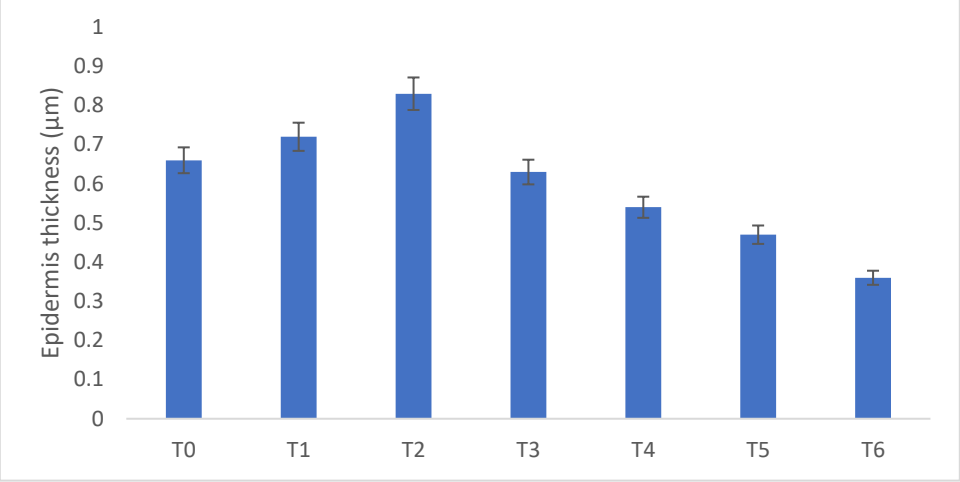

Figure 3.36: Comparison of epidermis thickness (µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

Fig. shows that the plant under treatment of NaCl in T_3 showed a 0.63µm epidermis thickness. The increase in thickness occurred when treated with minimum and maximum concentrations of Auxin at the same time, pre or post of the NaCl application. It was 0.67µm and 0.71µm in T_7 and T_{11} in which both Auxin and metal were applied at the same time, 0.69µm and 0.75µm in T_{15} and T_{19} in which metal was applied a day before the application of Auxin and 0.68µm and 0.72µm in T_{23} and T_{27} in which metal was applied a day after the application of Auxin respectively.

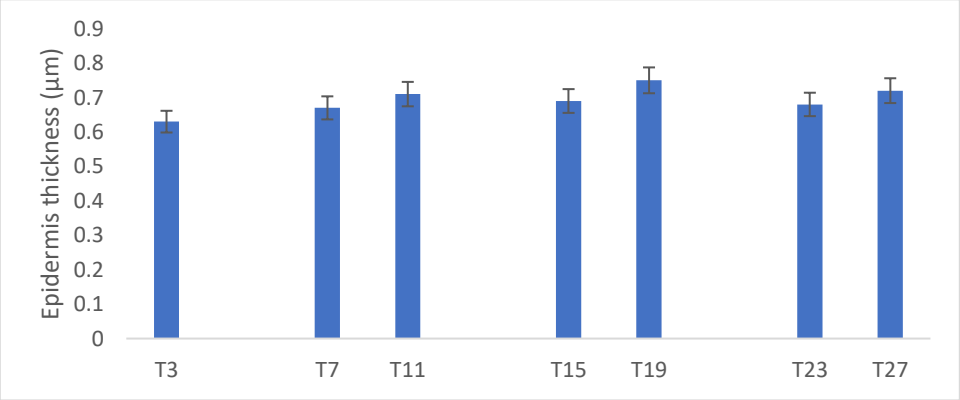

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Figure 3.37: Comparison of epidermis thickness (µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM NaCl), T11(0.5mM NaCl + 2.5mM IAA), T15(pre0.5mM NaCl + post 0.5mM IAA), T19(pre0.5mM NaCl + post 2.5mM IAA), T23(post 0.5mM NaCl + pre0.5mM IAA), T27(post 0.5mM NaCl + pre2.5mM IAA).

As shown in Fig Plants in T_4 had 0.54µm thickness, when IAA was applied at the same time with NaCl, pre or post of application of NaCl it showed an increase in thickness was 0.57µm and 0.6µm in T_8 and $T_{12,}$ in which IAA and metal were applied at Same time, a huge increase in thickness was observed when IAA was applied after the application of NaCl and it was 0.59µm and 0.65µm in T_{16} and T_{20}. While a slight increase was observed when IAA was applied a day after the application of NaCl it was 0.58µm and 0.62µm in T_{24} and T_{28} respectively.

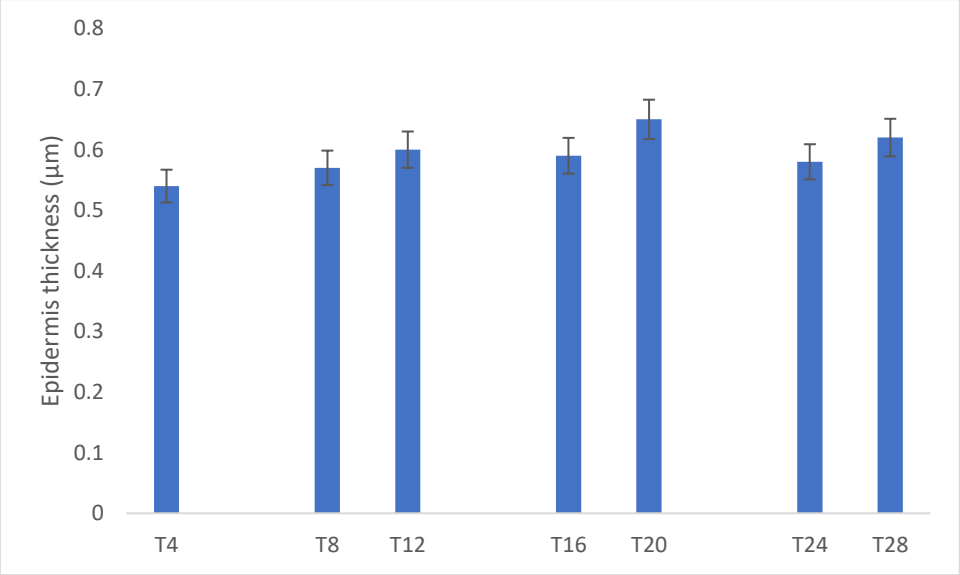

Figure 3.38: Comparison of epidermis thickness (µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time T4(1mM NaCl), T8(1mM NaCl + 0.5mM NaCl), T12(pre1mM NaCl + post 2.5mM IAA), T16(pre 1mM NaCl + post 0.5mM IAA), T20(post 1mM NaCl + pre2.5mM IAA), T24(post 1mM NaCl + pr 0.5mM IAA), T28(1mM NaCl + 2.5mM IAA).

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Fig. 3.39 shows that when plants were treated with sole NaCl in T_5 the epidermis thickness was 0.47µm and increase in thickness occurred when IAA was applied with NaCl, but when IAA was applied a day after the application of NaCl notable increase in epidermis thickness was observed. It was 0.5µm and 0.53µm in T_9 and T_{13}, 0.51µm and 0.57µm in T_{17} and T_{21} and 0.5µm and 0.54µm in T_{25} and T_{29} respectively.

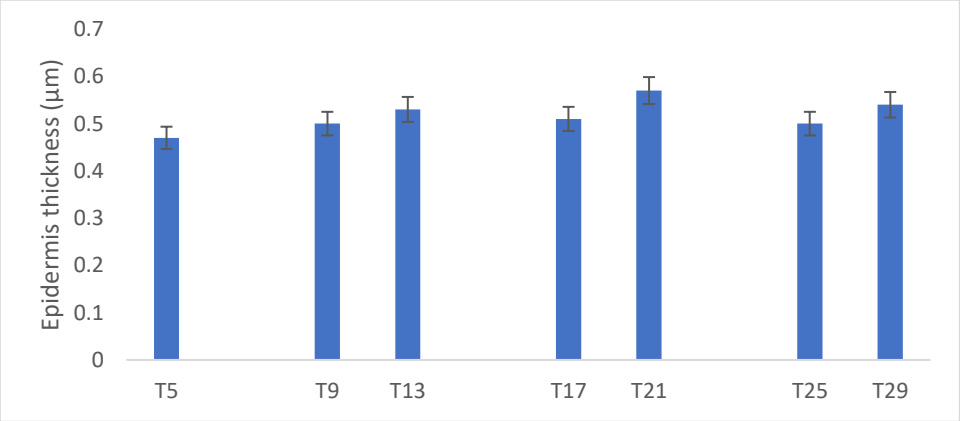

Figure 3.39: Comparison of epidermis thickness(µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T5(2mM NaCl), T9(2mM NaCl + 0.5mM NaCl), T13(2mM NaCl + 2.5mM IAA), T17(pre 2mM NaCl + post 0.5mM IAA), T21(pre 2mM NaCl + post 2.5mM IAA), T25(post 2mM NaCl + pre0.5mM IAA), T29(post 2mM NaCl + pre2.5mM IAA).

Fig. 3.4.0 shows that in T_6 minimum epidermis thickness was observed which was 0.36µm and it was slightly elevated with the application of both minimum and maximum concentration of IAA. Observed thickness was 0.38µm and 0.42µm in T_{10} and T_{14} in which both IAA and metal were applied at the same time, 0.4µm and 0.46µm in T_{18} and T_{22} in which metal was applied a day before the application of IAA while it was 0.39µm and 0.43µm in T_{26} and T_{30} in which metal was applied a day after the application of IAA respectively.

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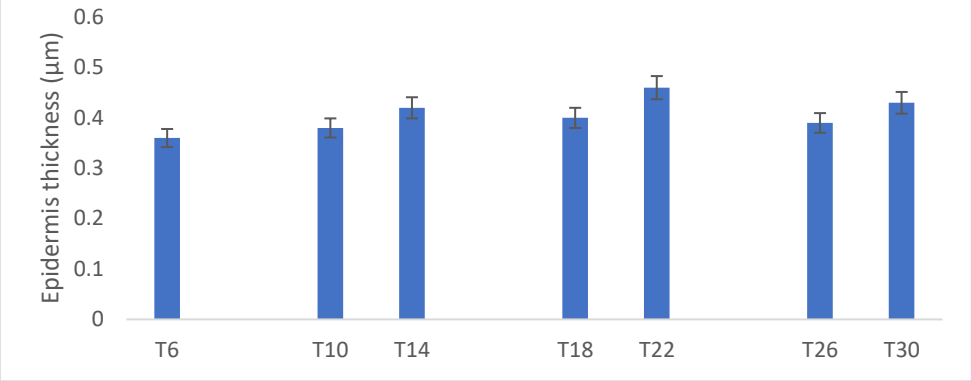

Figure 3.40: Comparison of epidermis thickness (µm) between different levels of Sodium chloride, Auxin, and Sodium chloride + Auxin when applied at different time

T6(3mM NaCl), T10(3mM NaCl + 0.5mM IAA), T14(3mM NaCl + 2.5mM IAA), T18(pre 3mM NaCl + post 0.5mM IAA), T22(pre 3mM NaCl + post 2.5mM IAA), T26(post 3mM NaCl + pre0.5mM IAA), T30(post 3mM NaCl + pre2.5mM IAA).

Plants to which maximum Auxin was applied showed maximum epidermis thickness. Treatments with less NaCl and both concentrations of regulator showed enhanced epidermis thickness as compared to the sole metal, but the treatments in which IAA was applied a day after the application of NaCl showed a notable increase in epidermis thickness. It is shown that maximum root epidermis thickness was observed in treatments in which IAA was applied exogenously a day after low to medium concentrations of NaCl.

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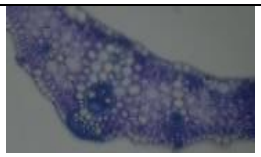
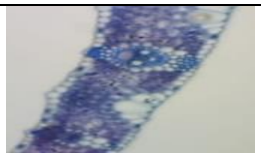
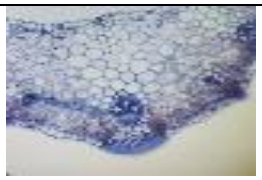
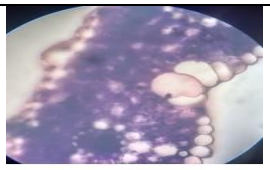
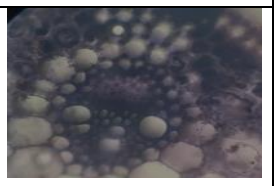
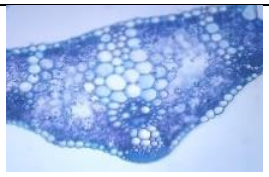
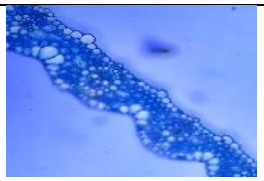
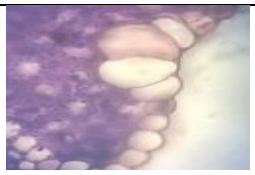
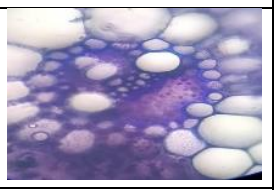
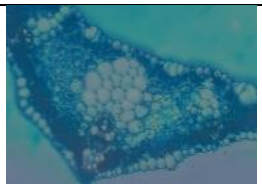
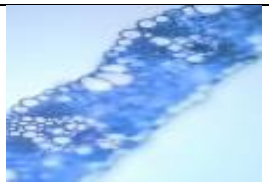
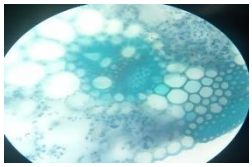
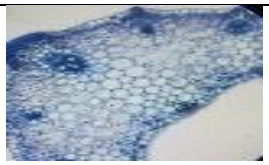
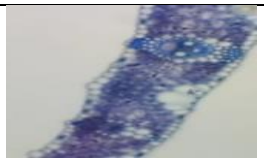
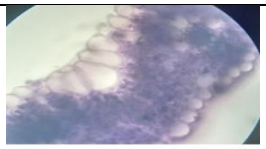
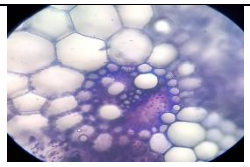
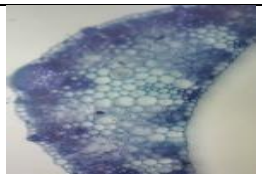
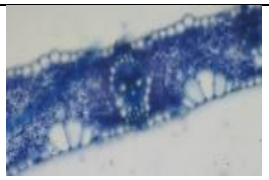
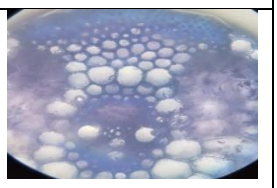
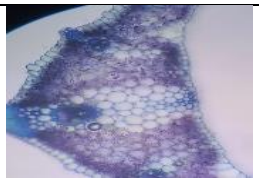
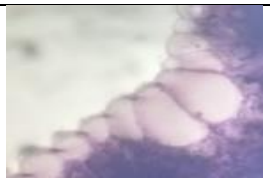
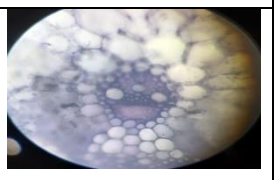
	4(X)	10(X)	40X B.	40X V. (Region)
T0				
T1				
T2				
T3				
T4				
T5				
T6				

Plate 3.1: T0 (Control), T1(0.5mM IAA), T2(2.5mM IAA), T3(0.5mM NaCl), T4(1mM NaCl), T5(2mM NaCl), T6(3mM NaCl).

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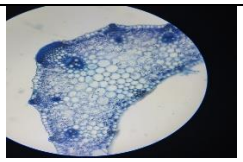
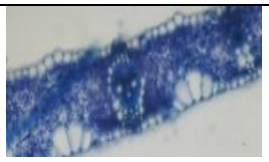
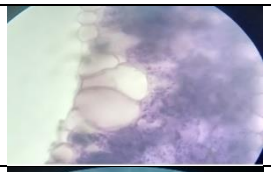
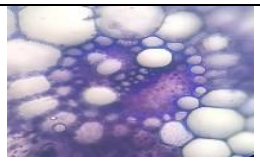
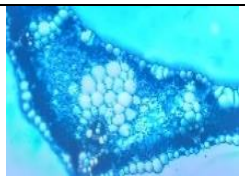
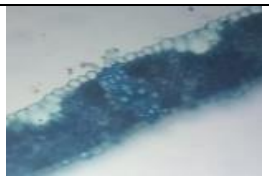
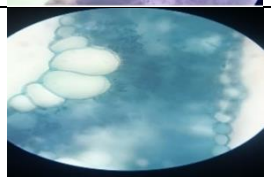
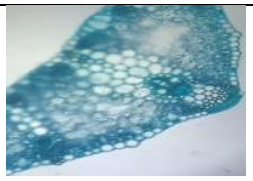
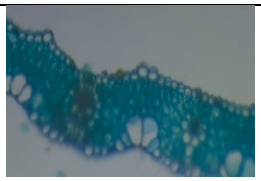
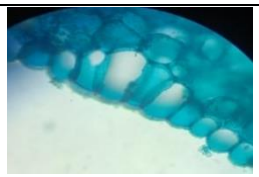
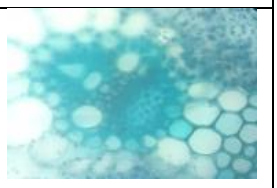
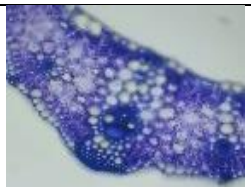
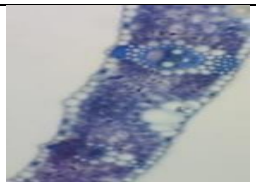
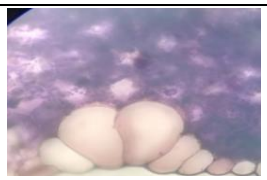
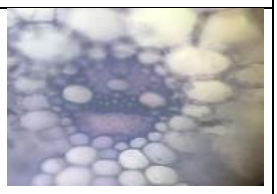
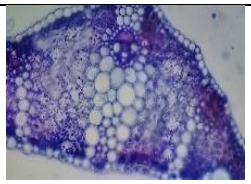
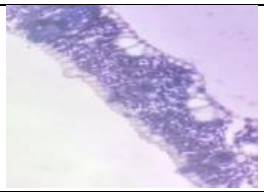
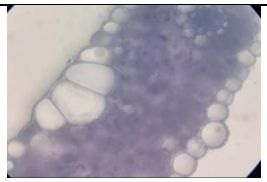
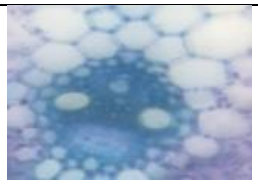
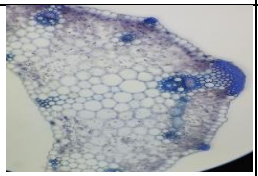
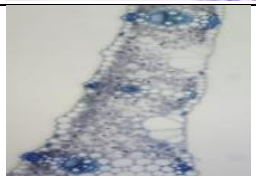
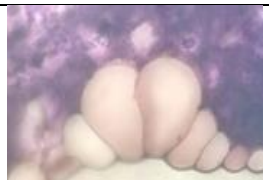
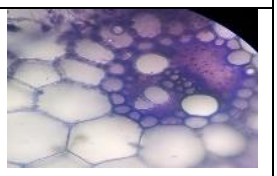
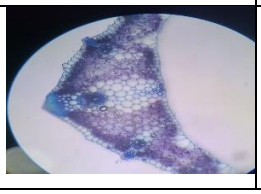
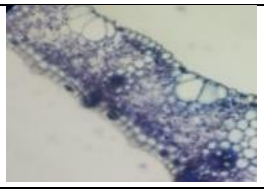
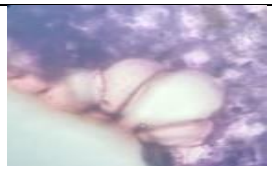
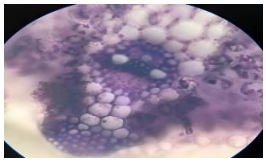
T3				
T7				
T11				
T15				
T19				
T23				
T27				

Plate 3.2: T3(0.5mM NaCl), T7(0.5mM NaCl + 0.5mM NaCl), T11(0.5mM NaCl + 2.5mM IAA), T15(pre0.5mM NaCl + post 0.5mM IAA), T19(pre0.5mM NaCl + post 2.5mM IAA), T23(post 0.5mM NaCl + pre0.5mM IAA), T27(post 0.5mM NaCl + pre2.5mM IAA).

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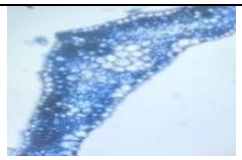
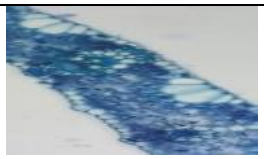
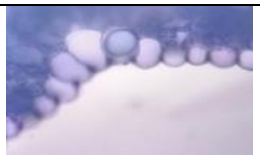
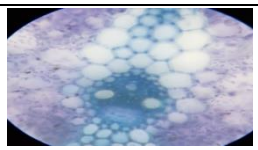
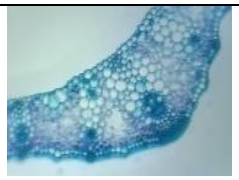
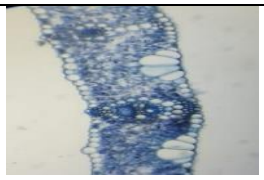
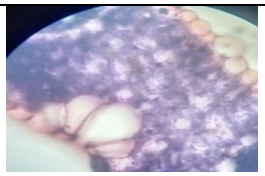
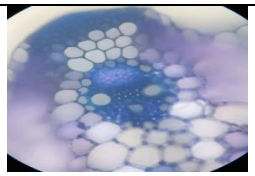
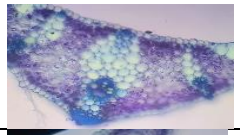
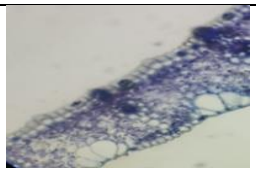
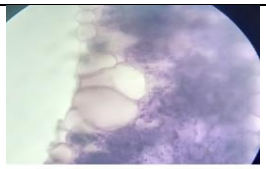
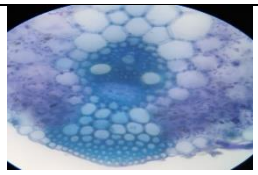
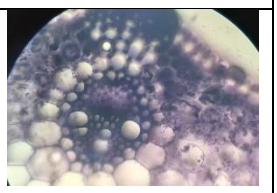
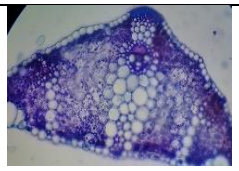
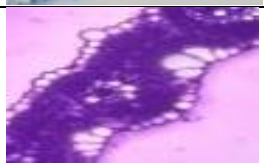
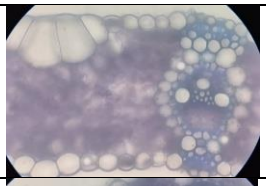
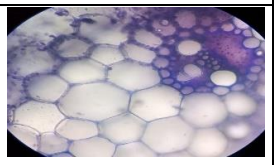
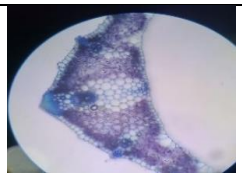
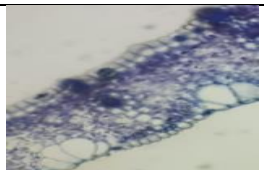
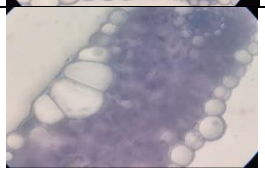
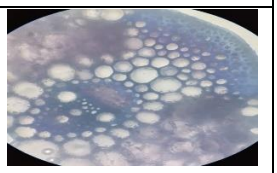
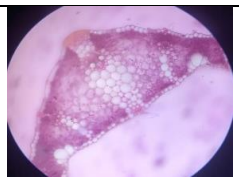
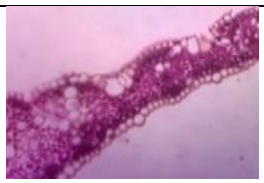
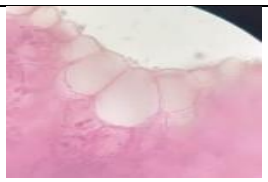
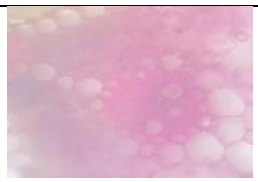
T4				
T8				
T12				
T16				
T20				
T24				
T28				

Plate 3.3: T4(1mM NaCl), T8(1mM NaCl + 0.5mM NaCl), T12(pre1mM NaCl + post 2.5mM IAA), T16(pre 1mM NaCl + post 0.5mM IAA), T20(post 1mM NaCl + pre2.5mM IAA), T24(post 1mM NaCl + pr 0.5mM IAA), T28(1mM NaCl + 2.5mM IAA).

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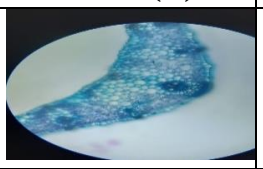
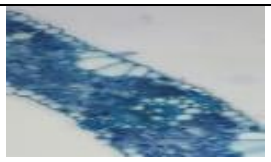
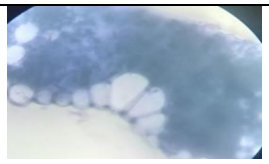
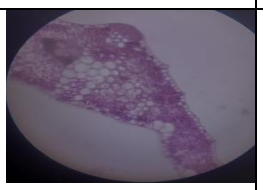
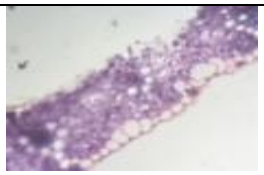
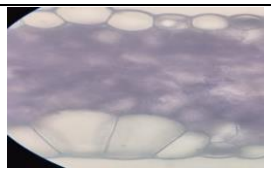
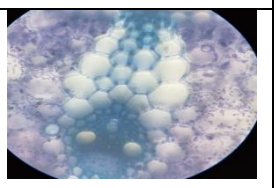
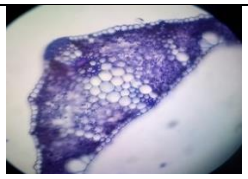
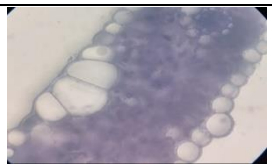
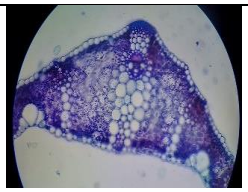
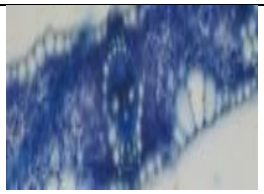
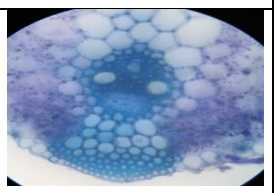
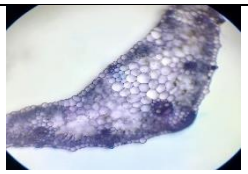
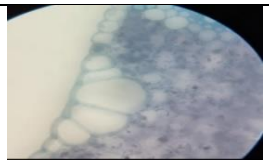
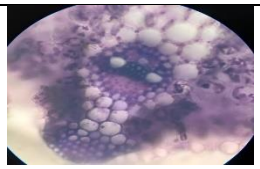
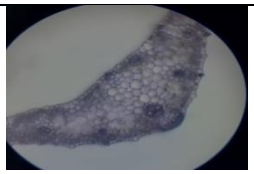
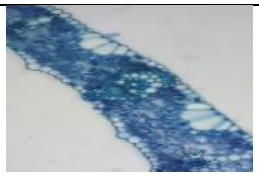
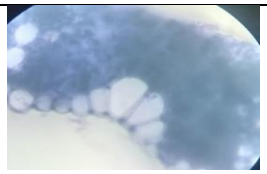
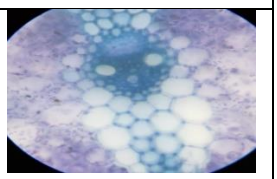
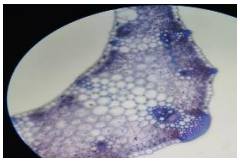
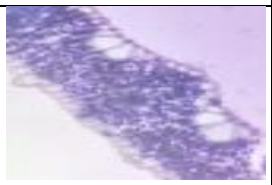
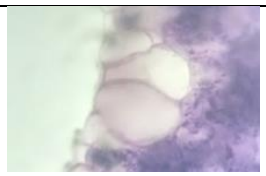
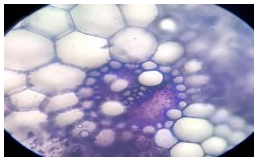
T5				
T9				
T13				
T17				
T21				
T25				
T29				

Plate 3.4: T5(2mM NaCl), T9(2mM NaCl + 0.5mM NaCl), T13(2mM NaCl + 2.5mM IAA), T17(pre 2mM NaCl + post 0.5mM IAA), T21(pre 2mM NaCl + post 2.5mM IAA), T25(post 2mM NaCl + pre0.5mM IAA), T29(post 2mM NaCl + pre2.5mM IAA).

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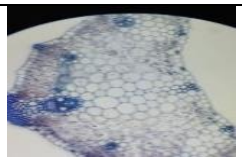
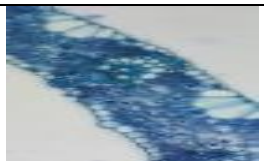
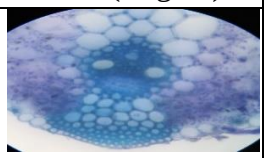
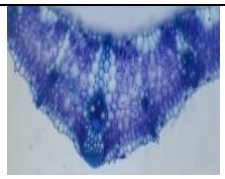
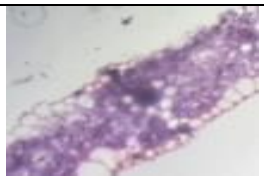
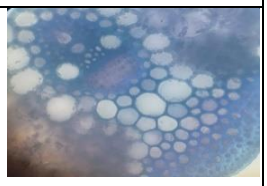
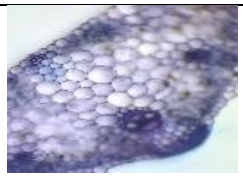
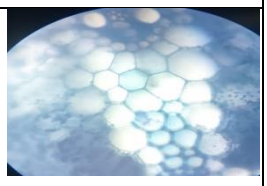
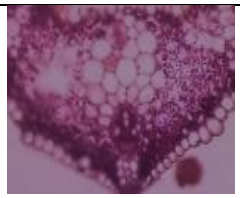
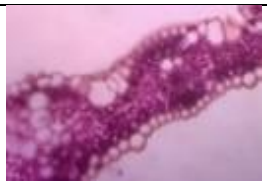
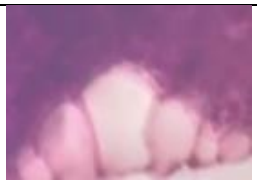
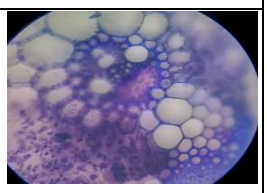
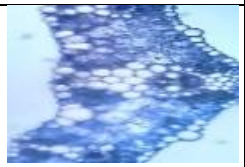
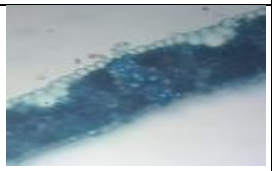
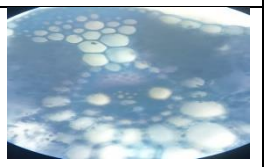
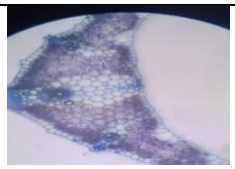
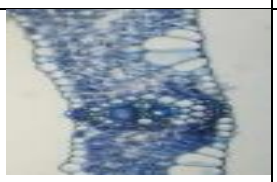
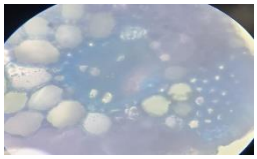
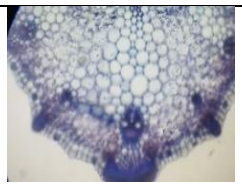
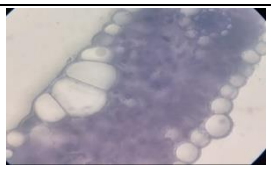
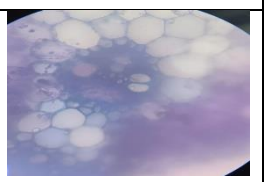
T6				
T10				
T14				
T18				
T22				
T26				
T30				

Plate 3.5: T6(3mM NaCl), T10(3mM NaCl + 0.5mM IAA), T14(3mM NaCl + 2.5mM IAA), T18(pre 3mM NaCl + post 0.5mM IAA), T22(pre 3mM NaCl + post 2.5mM IAA), T26(post 3mM NaCl + pre0.5mM IAA), T30(post 3mM NaCl + pre2.5mM IAA).

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4. Discussion

When Salt stress was increased in contrast to plants grown under controlled conditions, the fresh and dry weight of the plants dropped. Fresh and dried plant weights were shown to be considerably lower at high Salt stress levels (0.5, 1, 2, and 3 mM). (Tanveer & Wang, 2019) Examined the effects of excessive Salt on tomato plants. He found that salinity reduced the leaf area, the fresh and dry weight of the roots, the fresh and dry weight of the shoots, and the stability of the membrane.

(Smith et al., 2018) studied that both the fresh and dry masses of the root and shoot were significantly reduced at high NaCl solutions. In plants of Helianthus (Abed & Zeboon, 2020) said that all genotypes of kivi fruits displayed a decline in plant dry weight (PDW) and plant fresh weight (PFW) under saline stress. (Movafegh et al., 2012) evaluated that high Salt stress significantly lowers the percentage of seeds that germinate in several plant species, such as Hordem vulgare cultivars that affect dry weight. (Jamil & Rha, 2013) reported that the fresh weight of cotton (Gossypum hirsutum) and the growth properties of Brassica juncea or other plants are impacted by saline stress. (Balal, Cheu, & Sarkodie-Gyan, 2016) observed that Salt stress caused a decrease in the weight of the cotton seedlings, both fresh and dried.

Plant root and shoot length decreased by increasing the level of Salt stress in comparison to plants under controlled conditions. It was detected that plant root and shoot length significantly reduced at the high level of Salt stress (0.5mM, 1mM, 2mM, 3mM). (Mahjoor, Ghaemi, & Golabi, 2016) Found that plant height and root length were significantly reduced after being treated with Salt. (Kara, 2010) observed that the effects of NaCl concentrations caused a significant reduction in the root length of wheat plants(Jameel et al., 2024) the Bonita variety showed a maximum reduction in root length, shoot length, and number of

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roots while compared with other varieties. (Tanveer, Shahzad, Sharma, & Khan, 2019) Found that salinity reduced root and shoot length in tomato plants. With the application of IAA, plant growth increased as it makes the plant able to cope with mental stress this IAA work is also supported by (Steenackers, Parijs, Foster, & Vanderleyden, 2016) who stated that IAA affected the manufacturing and generation of secondary metabolites like lignin and flavonoids, which in turn-controlled plant structure and biomass accumulation. With the application of IAA plant growth becomes improved as a growth regulator it mitigates the effects of copper sulfate and enhances the growth of plants This work is also supported by (Hamid, Mir, & Rohela, 2020) who stated use of IAA foliar significantly enhanced all growth parameters (root and shoot length and as well as fresh and dry mass)

Reduced upper epidermis thickness, ground tissue thickness, lamina thickness, vascular bundle area, and midrib thickness are the results of increased Salt concentration in plants. Furthermore, endorsing his work is (Mikovilovi & Dragosavac, 2010). The top and lower epidermis, as well as the mesophyll cells of leaves, become thinner due to heavy metal exposure.In 2009 (Gratao *et al.*,) concluded that the smaller cells in the palisade parenchyma caused the Salt to trigger anatomical changes in the leaves, leading to a thinner leaf lamina. (Iqbal *et al.* 2020) concluded that the diameter of the xylem vessel components decreases with an increase in Salt concentrations. According to (Nazir *et al.*, 2021), Salt induces a decrease in a leaf's epidermis. With the foliar application of IAA, the leaf anatomical structures like leaf lamina, midrib thickness, upper epidermis, lower epidermis and ground tissue thickness increased the IAA work is also supported by (Di Benedetto *et al.*, 2015) which stated that plant treated with IAA the anatomical characteristics of leaf like lower epidermis, upper epidermis, midrib thickness and leaf lamina becomes start increasing.

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