# THESIS

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# Hungarian University of Agriculture and Life Science Szent István Campus MSc Crop Production Engineering

# **EFFECTS OF COMBINED TEMPERATURE, WATER, AND SALINITY STRESSES ON MAIZE** (*Zea mays*) **SEEDS GERMINATION AND DEVELOPMENT**

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#### 1. INTRODUCTION

Climate change, as defined by the United Nations, encompasses any major alteration to temperature and weather patterns that last for an extended time. These changes can be natural, like slight variations in the sun's cycle. Most people assume that global warming only refers to heated weather. However, this is far from the truth. Climate change has many different causes and consequences- all of which are interconnected. A few examples of climate change include droughts, water shortages, raging fires, melting polar ice caps, devastating storms, and plunging biodiversity levels.

Specifically, the world's food production systems are threatened by climate change, which could make many areas less suitable for growing crops. Climate change can have a positive or negative effect on crops, depending on the circumstances. In general, though, more droughts and floods could cause problems for farmers trying to maintain food safety (USGCRP, 2014). This would lead to an increase in hunger and poverty around the globe; it has been estimated that over a billion people depend on agriculture-based economies for their livelihoods. To ensure food security globally, it is essential to find solutions that allow crops to adapt to the changing climate and increase crop production.

Maize is a major crop grown for food and forage. It is the most extensively produced crop globally (Ali et al., 2022). That is because maize can be grown in a wide range of soil and climatic conditions around the world. However, they tend to be the most vulnerable to the effects of climate change in agriculture (Tebaldi & Lobell, 2018).

In the tropics, maize is largely grown as a rain-fed crop in marginal areas that are often facing various biotic and abiotic stresses such as drought and waterlogging at the same time. This leads to stunted plant growth which can affect both nutritional contents for humans who consume it or animal feed from these plants - leading down the productivity chain (Rafique et al., 2020). And with the pressing effect of climate change in the world, can potentially impair maize production and productivity.

Many factors can affect the growth and development of plants. These include drought, waterlogging (or becoming too wet), high temperatures, or salinity in their environment throughout the life cycle stages from germination through maturity. These environmental stresses are projected to even intensify and become more common in the years to come. These stresses are the major constraints limiting the production of maize worldwide.

It is a reality that agricultural regions around the world are commonly stricken by two or more stresses at once, such as drought and high salt content in the soil, heat and high salt content, or extreme temperature paired with little rainfall (Yang et al., 2022). When two or more abiotic stresses are combined, the effect of these stresses individually will be more pronounced, forcing farmers to spend more on input to at least lessen the negative impacts. Relatedly, according to Jolánkai et al. (2018), the interaction between water and temperature can determine the quantity and quality of crop yield and performance. In addition, heat stress reduces root biomass and growth by altering the number and elongation of lateral roots in plants. Roots have direct contact with the soil, hence, they are the primary sensors for heat stress and other types of abiotic stresses. (Davies & Bacon, 2003) According to Hall (2000), heat stress is due to high ambient temperatures which pose a serious threat to crop productivity worldwide.

Water-logging, another type of abiotic stress, happens when there is too much water which fills the tiny pores in the soil and causes a drastic drop in oxygen levels near plant roots. This also decreases activity among aerobic (oxygen-loving) microbes living in the soil (Tian et al., 2019). If waterlogging in the soil is further continued, it would result in the anoxia of crop roots, and inhibition of root respiration (Malik et al., 2002). This would roll into stomatal closure, reduction in transpiration rate, and photosynthetic rate until finally impacting crop yield. In maize production, continued waterlogging can cause a reduction in grain yield due to decreased ear length and width.

Salinity stress is typically caused by the high concentration of NaCl in the soil, which induces abiotic stress in plants. According to a global estimate, 20% of cultivated land and 50% of irrigated land are under salinity stress (Wang, 2017). Plants are affected by salinity stress through their plant growth and productivity. This is because due to osmotic stress and ion toxicity. As an effect, osmotic stress causes a decrease in stomata opening and a reduction in the photosynthetic ability of the plant (Munns & Tester, 2008).

Moreover, salinity stress can also contribute to secondary stress. One example is oxidative stress which can damage plant cells by excessive accumulation of reactive oxygen species. Oxidative stress is mainly caused by ion toxicity and osmotic stress as well, both discussed earlier (Chaves, Flexas, and Pinheiro, 2009).

In plants, drought stress inhibits both the initiation and elongation of lateral roots. The primary root continues to grow longer under these conditions to reach water sources deeper in the soil so that the seedlings can establish themselves before shoot emergence (Xu et al., 2013). However, researchers have also shown the ability of plants to respond quickly and efficiently as one of their most impressive features. They have developed several mechanisms for detecting environmental changes, such as water stress or stronger light levels--and these skills allow them not just to survive but thrive under challenging conditions.

According to Rizhsky et al. (2004), plants have evolved to deal with a combination of multiple abiotic or biotic stresses by activating a specific and unique stress response. These responses are modified in such a way that can be unique or common, depending on the combination of the multiple stresses affecting them. On the other hand, the damage caused by combined drought and heat stresses to crops is more severe than their separate occurrences at different growth stages. (Prasad et al., 2011)

In this regard, the plant-stress interaction has been the subject of years of studies. However, most were focused on a single stressor at a time which made it difficult to conclude how various factors when combined might affect a crop (Mittler, 2006). Although in maize, for example, according to Jiang and Huang (2001), some breeding strategies emphasized the tolerance of the crop to two different abiotic stresses at the same time.

Abiotic stress is a premier challenge to farmers looking to maintain increases in crop production with ever-changing climate patterns. Yield, plant characteristics, and yield potential are all largely determined by environmental conditions and the plant's genotype (Mao et al., 2015).

It is important to determine the significance of the effect of combined abiotic stresses on maize germination because according to recent research, there are plants that respond differently to multiple stresses and individual stresses. Meanwhile, with the climate change that has prevalent effects on crop production these days, there is not only one individual abiotic stress present to affect crops in the field, there are usually two or more.

Hence, it is important to uncover the mechanism of maize tolerance to combined abiotic stress. Specifically, there is a need to determine the effects of combined abiotic stresses such as temperature, water-logging, and salinity on maize seed germination. The results of this study will hopefully provide additional information to the development of abiotic stress-tolerant varieties of maize, which in turn will be significant in maintaining crop productivity around the world in the future.

#### 2. LITERATURE REVIEW

This chapter consists of a review of related literature and studies, essential to provide insights into the existing literature and research that were conducted on the topic of the effect of combined abiotic stresses on the germination of maize seeds.

#### 2.1. Maize Cultivation and Production around the World

Maize (Zea mays), also known to others as corn is a type of cereal crop cultivated around the world for a variety of food and industrial purposes, making it a versatile multi-purpose crop. Together with wheat and rice, it is among the important sources of human food, accordingly, from 2016 to 2018, accounting for an estimated 42% of the world's food calories and 37% of protein intake (FAO, 2021). In relation, it is the preferred cereal in Central America, Mexico which is believed to be where maize originated from, and Southern and Eastern Africa. With approximately 717 million metric tons/year produced, the United States, China, and Brazil are the top-producing countries of maize. (Ranum, Peña-Rosas, & Garcia-Casal, 2014)

In terms of their purpose for human consumption, maize flour, and maize meal are two of the most popular food products. Maize has about 72% starch, 10% protein, 4% fat, and an energy density of 365 kcal/100g (Nuss & Tanumihardjo, 2010). It also provides B vitamins among many other essential minerals and is used to improve micronutrient intake and prevent iron deficiency in countries with high cases of anemia and iron deficiency through iron and specific vitamins and minerals fortification in flour and cornmeal (WHO et al., 2009).

However, maize being a multi-purpose crop is also used as a feed crop for livestock and as an industrial crop. As livestock feed, the by-products in maize production provide a pathway of indirect consumption for animal-derived foods. According to Mottet et al. (2017), 3 kilograms of maize grain and soy can potentially produce 1 kilogram of bone meat, proving its significance aside from its purpose for direct human consumption. As an industrial and energy crop, maize serves as the primary element for ethanol production. In relation, since there is an upward demand for ethanol production, the same case can be said as well for maize production. It then contributes to maize prices and cultivated area for the crop, but on the other hand, opened up the discussion on the debate of "food versus fuel" (Committee on World Food Security. High-Level Panel of Experts, 2013).

On a related note, maize has different types, cultivated throughout the world, and ranges from yellow to white to red and black colors. Specifically, yellow maize is mostly cultivated in the United States meanwhile white maize is preferred in countries in Africa and Central America. The preference for the type of maize is usually because of tradition but in African countries, white maize is preferred because the yellow variety is seen as consumed only by poor people and is associated with food-aid programs (National Chamber of Milling, South Africa, 2008). However, yellow maize has higher concentrations of B-carotene, B-cryptoxanthin, and vitamin A precursors compared to the white variety. This means that since people often consume white maize, they have a lower intake of the aforementioned nutrients which is important nonetheless for the body.

Aside from the different colors of maize, the size and composition of the endosperm is also another classification that is usually taken into consideration. The categories under it are as follows: waxy, flour, sweet, dent, pop, flint, and pod corn. Sugar content in maize is also categorized, for example, sweet varieties are usually eaten fresh, frozen, or in the can because they cannot be stored for a long period and they are not also feasible to be fortified with vitamins and minerals (Gibson and Benson, 2002).

There is also genetically-modified maize, which has become popular enough to be a major type of maize cultivated in many countries because of its resistance to certain pests and diseases. They don't have any nutritional differences from other varieties of maize, hence, more and more countries are accepting the idea of cultivating them as well (Bouis and Welch, 2010). Maize has since become the second most widely grown genetically-modified crop worldwide with 32% cultivated GM area, just below soybean. Insect-resistant based in Bacillus thuringiensis and herbicide-tolerant maize are two of the most used GM variety of the crop, because of their cost-savings benefit and yield gains (Brookes and Barfoot, 2020).

In Hungary, maize is considered as one of the two major agricultural crops together with wheat. In 2020, the share of maize in terms of utilized agriculture area is almost the same with wheat and is roughly double as those of sunflower. Maize is an important production of the Hungarian agriculture, and due to land abundance, favorable weather condition, and significance production experience of Hungarian farmers, these allows for a significant trade surplus (Mizik and Rádai, 2021). Moreover, the first hybrid maize developed in Europe was the achievement of a Hungarian breeder, Endre Pap in 1953, recognized as a landmark achievement of plant breeding in Hungary (Marton, 2013).

#### 2.2. Maize Germination

Maize is a C4 plant with excellent photosynthetic efficiency. Because of this, it can perform well in a range of environments, may it be tropical, subtropical, or temperate zones (Mekonnen & Gerbens-Leenes, 2020). This makes them more favored to produce compared to wheat and rice. However, due to climate change and the pressing effects of global warming, yield reductions in maize production caused by drought and flood still occur. In fact, environmental stress is one of the main factors causing decline in photosynthetic activity. This will lead to weaker shoot growth and smaller green mass, ultimately affecting yield and quality of maize production (Széles and Nagy, 2012).

Seed germination is a fundamental process in crop production in which the plant grows from a single seed to a mature plant. It influences both the production of crops and its quality upon harvesting. Specifically, as discussed by Riley (1981), it is the increase in the activity of enzyme systems together with the production of new enzymes. It effectively uses nutrients and water resources and is essential in such a way that it determines the quality and quantity of the yield in a certain planting period (Gan, Stobbe, and Njue, 1996).

The process of germination starts with imbibition, when the seeds, at an optimum temperature, absorb water quickly that it swells and softens the seed coat. As the seed coat will rupture, it will lead to the emergence of the radicle within 2 to 3 days. The shoot will also come out and elongate. Then, the lag phase of seed germination happens, when the seed activates its internal physiology and respires (Gallardo et al., 2001). As the radicle grows out of the maize seed's coating layers and the coleoptile protrudes, germination ends. (Khalid et al., 2021)

Throughout this process, certain elements such as temperature, water, and oxygen are essential for healthy germination. For example, temperature is among the factors necessary for germination because it encompasses all individual reactions and stages (Bailly, 2019). When the temperature is at optimum, germination period is at its shortest time possible. In addition, water availability and temperature level significantly affect the biological and biochemical enzyme activities in stages of germination and seedling growth of plants (Khan et al., 2021). Moreover, oxygen also plays a vital role to initiate germination of plants. According to Xue et al. (2021), water level and oxygen availability in the soil is inversely proportional, in such a way that when water level goes beyond the optimal range, oxygen availability decreases.

Other factors that can influence seed germination as well are nutrients, sugar, and phytohormones (Xue et al., 2021). The environment also plays a role, in such a way that when the soil is either too warm, too wet, too dry, or too salty, the germination process may be slow or the germinated seeds may be negatively affected, resulting in death before it even develops fully (Edwards, 2009).

#### 2.3. Abiotic Stresses to Maize Germination.

There have been a lot of studies on the effect of abiotic stresses on crops, including maize, and works of literature can attest to that. However, these abiotic stresses tend to occur in combination in field-given conditions instead of individually, say salinity and water-logging

(Suzuki et al., 2014). And when these abiotic stresses occur together in a specific field, they often cause more damage to crops, may it be during the early stages of plant development or during flowering time, and even seed production. In fact, according to Matiu et al (2017), the yield of wheat and maize globally has already been reduced due to high temperatures but was even more intensified with the drought that came along. This section will discuss the three abiotic stresses that were included in this study, namely, temperature, water-logging, and salinity.

#### 2.3.1. Temperature

According to Mondo, Diaz, and Cicero (2015), temperature is one of the major abiotic factors that influence the growth, development, and yield of maize. In fact, plants may be exposed to various forms and combinations of drought stress, such as the period and starting date of the phenomena, the quantity and distribution of rainfall, and the intensity of solar radiation. In this case, according to Spitkó et al. (2014), to be able to predict drought tolerance in maize production, proterandry should be treated as a priority trait.

Specifically, during the development period of the crop, it needs a minimum temperature required to complete a specific phonological phase. Relatively, maize from late emerged seedlings needs longer cycles than those that developed from early emerged seedlings.

Maize, which originated from Central Mexico, is naturally sensitive to the stress of low temperatures, especially during seed germination, when produced in temperate areas (Zhang et al. 2020). Specifically, maize is vulnerable to this type of abiotic stress during the germination stage and the early stage of seedling establishment. Accordingly, even if there is already an increase in temperature, seedling growth is still affected due to its inability to respond quickly to favorable environmental changes, which is in this case the change from low to moderate or high-temperature levels. (Sowiński et al., 2005)

The minimum temperature for maize seed germination is approximately 10°C and irreversible damage to cells and tissue occurs when the level further drops. This will lead to the maize seeds not germinating at all and the halting of the growth of seedlings (yyGreaves, 1996, Li et al., 2018). It is important to determine the minimum temperature level for maize germination because the stress of low temperatures can decrease the emergence rate of the seeds and their vigor. In addition, it can also increase the chance of pathogenic infection by bacteria in the soil, in turn, can reduce the overall maize yield (Zhang et al. 2020).

On the other hand, according to a study by Khaeim et al (2022), the optimum temperature level for seedling growth is 20°C. However, the range of 20-30°C is ideal for maize seeds to initiate germination. Lower than this temperature range will lead to a decrease in

germination rate. Meanwhile, above this temperature range, maize seedlings will have poor growth because of temperature sensitivity in the embryo (Riley, 1981) and will also increase the occurrence of fungal growth. Maize seeds cannot germinate at a temperature level greater than 45°C (Sánchez, Rasmussen, and Porter, 2014). Radicle emergence starts after 24-36 hours when the temperature level is at 28°C.

#### 2.3.2. Waterlogging

In the past years, the climate around the world has become increasingly unpredictable leading to frequent extreme weather events. One of those weather events is extreme precipitation which lead to waterlogging stress, further heightened by poor soil drainage. According to Shabala (2011), waterlogging affects 12% of cultivated areas globally and causes up to 20% of yield loss in agriculture. It causes detrimental effect on agricultural production, specifically from seed germination to vegetative and eventually reproductive growth. The negative effects of waterlogging on crops are not only because of prolonged periods but also under very short periods such as hours or days (Arduini et al., 2016).

When waterlogging occurs in an area, the soil pores become saturated with excess water which leads to an increase in the fast drop of the oxygen level in the root zone (Tian et al., 2019). Waterlogging, according to Arduini et al. (2016), in general, brings changes to the physiological and morphological aspects of plants. In addition, the performance of plants as a response to the stress of waterlogging is dependent on the plant growth stage, the duration of waterlogging in the soil, and the exact depth of the water level (Malik et al., 2001). But, at the seedling growth stage, water stress significantly decreases the vigor of the seedlings, so at the optimum range, seedling length will increase positively. (Khaeim et al., 2022)

For maize, a study by Yu et al. (2015) suggested that the root growth of summer maize has been negatively affected by water-logging stress which ultimately leads to a reduction in shoot growth. Specifically, waterlogging for four (4) days during the early growing stage of maize can limit seedling growth, among others, including reduced ear and internode length, stem diameter, and even plant height. (Azahar et al., 2020) To support this, a study by Huang et al (2022), showed that the height of maize is negatively affected by waterlogging at the seedling stage and it is directly proportional to the increase in the duration of waterlogging.

#### 2.3.3. Salinity

Salinity is defined as the buildup of significant soil levels in soil and water. According to Athar and Ashraf (2009), approximately 45 million hectares of the 230 million hectares of irrigated agricultural land worldwide have significant salinity level that affects their productivity. This is significantly increasing, however, because of the ever-changing climate

scenario and global warming. In fact, the presence of high salt content in the soil is among the main environmental factors that limit crop production in the world today with sodium chloride as the most common salt in saline soils (Zörb et al., 2004).

Its effect on the plant is manifested through a decrease in the relative water potential of plants, causing a decrease in the growth of the plants (El Sabagh et al., 2020). In addition, according to Adnan et al (2020), salinity stress can also lead to a decline in soil and water quality both in the short and long term duration and is associated with moisture stress in the soil. Even when soil moisture is not limiting crop productivity, salinity stress causes a decrease in plant growth and reduced plant yield (Sabagh et al., 2019).

As a C4 crop with a relative tolerance against salinity, maize can grow in both saline and non-saline conditions. However, the length and severity of the salinity stress on maize can still negatively affect the growth and yield of the crop, together with the phase of the crop during which the stress happened. The latter is during the initial growth stage of the crop when maize is highly sensitive to stress (El Sabagh et al., 2021). According to Taiz and Zeiger (2003), this is due to the reduction in the osmotic potential in the soil that prevents the entry of water into the maize seed. The entry of sodium and chloride ions during the development of the seedling causes toxicity in the plant cells, and in turn, decreases the rate of germination together with the growth of the seeds that have already germinated (Carpýcý, Celýk, and Bayram, 2009)

Aside from decreased germination rate, salinity stress during the initial growth stage can also delay the germination process as a whole, which in turn, adversely affects the seeds that have already germinated and reduces their survival rate. It happens when the oxidative stress is increased through the absorption of Na+ in the seeds which is damaging overall in maize germination. With this, it is important that at the early stage of the development of maize, recognizing the harmful impact of soil and water salinity is important to avoid a drastic reduction in crop productivity and yield.

#### 2.3.4. Combined Abiotic Stresses to Maize Germination

Plants are always subjected to various environmental stresses in field conditions anywhere in the world. These stresses range from temperature, drought, waterlogging, salinity, and even metal toxicity. Each one of these affects the plant's growth and development at any stage, but more effective during the development stage (Javed et al., 2020, Nováky, 2007). In fact, maize yield are always significantly influenced by the crop's resistance to abiotic stress, and that includes chilling temperature levels in an early spring. (Marton, 1990) However, in field conditions, these abiotic stresses do not come alone. They frequently affect the crop as a combination of two or more stresses, creating a more negative impact compared to any of them individually (Jolánkai et al., 2018). For example, the interactions between water and temperature determine the yield and quality performance of most crops. (Jolánkai et al., 2018b)

Salinity stress can occur in waterlogged soils, as well as salinity during drought, while high-temperature stress simultaneously appear with salt stress, especially in semiarid and arid regions in the world. The stress of high temperature and salinity stress on the plants causes more negative effect to the germination rate than those plants with saline soil but with optimum temperatures (Shahid et al., 2017). All of these combinations of abiotic stresses negatively affect crop performance and yield (Duan et al., 2018). As a matter of fact, the combination of these abiotic stresses can reduce the germination rate, shoot length, and dry weight of seedlings, forcing farmers to spend more on inputs to be able to reduce the impact of it on the yield performance of crops.

#### 3. MATERIALS AND METHODS

- **3.1. Laboratory Experiment.** The experiment for the study was conducted in the laboratory of the Institute of Agronomy at the Hungarian University of Agriculture and Life Sciences (MATE) in Gödöllő, Hungary. To reiterate, the objective of the study is to determine the effects of combined abiotic stresses (temperature, waterlogging, and salinity) on maize germination. During the experiment, the following materials were utilized:
  - Transparent Petri Dishes;
  - Micro Pipette;
  - Filter Papers;
  - Distilled Water;
  - Salt (NaCl);
  - Climate Chamber;
  - Precision Scale; and,
  - Maize Seeds.
- **3.2. Maize Seeds Used.** The experiment was conducted by general laboratory standards. Seed samples of horse-tooth maize were used and tested for their germination rate, seedling, radicle, and shoot growth under combined abiotic stress conditions. The experiment had three replications in a Memmer-type climatic chamber.
- **3.3. Treatments.** During the experiment, the germination of maize seeds was tested under three combined abiotic stresses. These treatments were set in a randomized complete block design (RCBD) with twenty-seven (27) treatments (as shown in Table 1) and three (3) replications for each factor. The Petri dishes used have all the same size with a diameter of 90mm. In relation, a single layer of filter paper was used for the experiment, which has the same size as the petri dish.

Temperature °C (T)	Water level ml (W)	Salinity level ppm (S)	Treatment Number
	6 ml	0	(1)
20	9 ml	1000	(2)
	12 ml	1500	(3)
	6 ml	0	(4)
25	9 ml	1000	(5)
	12 ml	1500	(6)
	6 ml	0	(7)
30	9 ml	1000	(8)
	12 ml	1500	(9)

Table 1. Treatment specifications of the experiment.

**3.4. Manipulation.** The researcher used healthy horse-tooth maize seeds for the experiments. Ten (10) seeds were allotted for each Petri dish, which was treated with fungicide earlier to eliminate pest factors to the germination of maize seeds. A total of eighty-one (81) Petri dishes were used for the treatments. The experiment was conducted during the second to the fourth week of October and was done in batches. The first batch was the treatments belonging to the 20°C and 25°C temperature levels combined with varied water and salinity levels, indicated in the experiment. These treatments were then observed for a period of seven (7) days, excluding weekends since the laboratory is closed. The same was done for the second batch, with the treatments belonging to the 30°C temperature level combined with the indicated levels of water and salinity in the experiment. During the entire experiment period, the researcher measured the amount of germinated seeds, seedling rate, and the growth of radicle and shoot of each germinated maize seed.

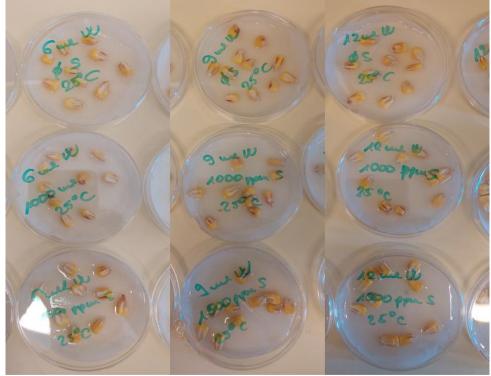


Figure 1. Maize seeds in petri dishes during the start of the experiment.

- **3.5. Collection of the Results.** As mentioned in the earlier section, data was collected from each petri dish every day for a period of seven days (excluding weekends) on the following parameters:
  - Amount of germinated seeds;
  - Length of the radicle (in mm); and,
  - Length of the shoot (in mm).



Figure 2. Maize seeds under combined abiotic treatments after 48 hours.



Figure 3. Maize seeds under combined abiotic treatments after 72 hours.



Figure 4. Maize seeds under combined abiotic treatments after 96 hours.



Figure 5. Maize seeds under combined abiotic treatments after 144 hours.

**3.6. Data Analysis.** After the collection of the raw data from the experiment conducted, the research applied a one-way analysis of variance to test the significant difference between each combined treatment used. Since all of the treatments bear significant differences amongst each other, the researcher proceeded to determine which

treatments are statistically different through Fisher's least significant difference test, under the standard significance level of p < 0.05.

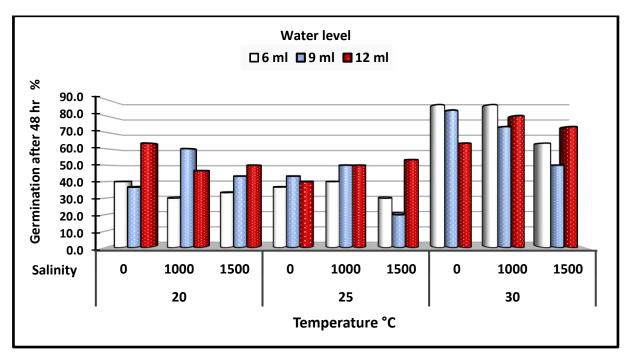
#### 4. RESULTS

This section of the study shows the main result and discussion of the analyzed recorded data, presented in tables and figures for easy understanding by the readers.

#### 4.1. Germination Rate Analysis of Maize Seeds.

In this section, the germination rate analysis of maize seeds will be presented, focusing on which combinations of temperature, water, and salinity resulted in the highest and lowest germination rates, across different time durations.

Presented in Figure 3 is the germination rate of maize seeds after 48 hours. Accordingly, two combined treatments resulted in the highest rate of maize seeds germination at 86.7 percent. The first combined treatment is 6 ml of water level, 0ppm of salinity, and a temperature level of 30°C. The other combined treatment is almost the same with 6ml of water level and temperature level of 30°C but with 1000ppm salinity level. This signifies that an increase in the salinity level of the treatments from 0-1000 ppm does not negatively affect the germination rate of maize seeds. However, at the maximum level of 1500ppm, maize germination rate can be reduced which is supported by the result of the combined treatment with the lowest germination rate at 20 percent (9ml water, 1500ppm salinity, and 25°C temperature level).



#### Figure 6. Germination rate of maize seeds after 48 hrs.

Figure 4 presents the germination rate of maize seeds after 72 hours. The combined treatment of 9ml water level, 1500ppm salinity level, and 20°C water level reached the highest germination rate with 90.0 percent. Meanwhile, the lowest germination rate for the maize seeds

after 72 hours was 56.7 percent from the 6ml water level, 1500ppm salinity level, and 25°C water level of combined treatment. From this result, it can be analyzed that lower water levels at this point can pose negative results to the germination rate of maize seeds. However, a high salinity level does not necessarily result from either a low or high germination rate.

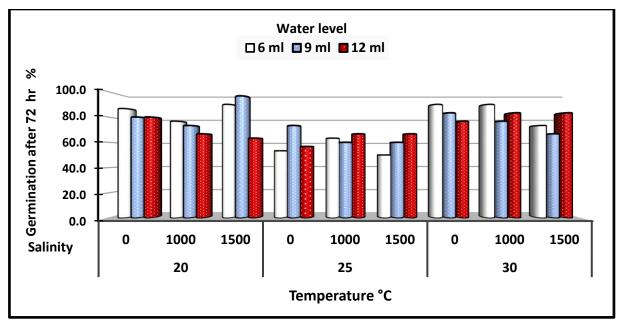


Figure 7. Germination rate of maize seeds after 72 hrs.

In Figure 5, after 96 hours of maize seeds under various combinations of treatments, one reached 96.7 percent of germination rate, 9ml of water level, 1500ppm of salinity level, and 20°C of temperature level. The lowest germination rate from the batch is 60.0 percent from a combined treatment of 9ml water level, 1000ppm salinity level, and 25°C temperature level. With these results, it can be deduced that an increase in the temperature level can be a contributing factor to the reduction of the germination rate of maize seeds. This is supported by a study by Edwards (2009) where accordingly, the environment also plays a role, in such a way that when the soil is either too warm, too wet, too dry, or too salty, the germination process may be slow or the germinated seeds may be negatively affected, resulting to death before it even develops fully.

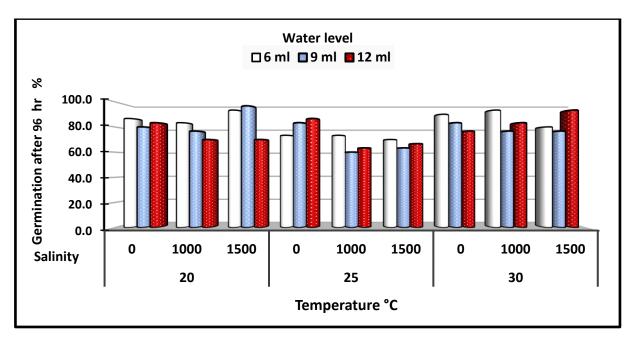


Figure 8. Germination rate of maize seeds after 96 hrs.

In Figure 6, the germination rate of maize seeds after 144 hours was presented. One combined treatment reached the full 100 percent germination rate: 12ml water level, 1500ppm salinity level, and 30°C temperature level. Other combined treatments that have reached a notable rate of 90 percent and above are as follows: 9ml water level, 1500ppm salinity level, and 20°C temperature level (96.7 percent), 6ml water level, 1500ppm salinity level, and 20°C temperature level (93.3 percent), 6ml water level, 1000ppm water level, and 30°C temperature level (93.3), 6ml water level, 0ppm salinity level, and 20°C temperature level, 0ppm salinity level, and 20°C temperature level, 0ppm salinity level, and 20°C temperature level (90.0 percent), and 6ml water level, 0ppm salinity level, and 30°C temperature level (90.0 percent). The result agrees with the findings of Khalid et al. (2021) where they found out that there is a strong correlation between salinity levels and germination rate of maize seeds.

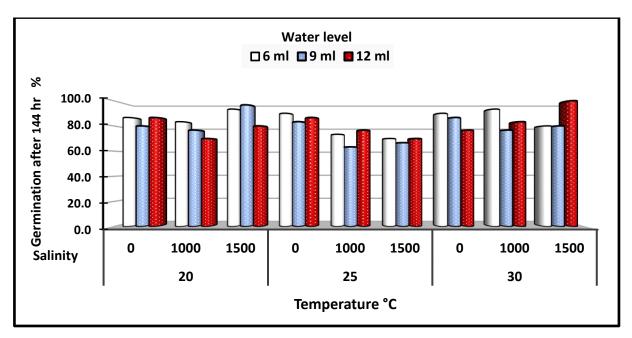


Figure 9. Germination rate of maize seeds after 144 hrs.

Based on the table below (Table 2), the germination rates of treatments with variance in temperature and salinity levels have significant differences from each other. In terms of the effect of combined temperature and salinity levels on the germination rate of maize seeds, the results showed that the higher the temperature is, the germination rate will also be higher. However, although there is not much difference, the results also showed that the germination rate decreases as the temperature and salinity level increase. It can be deduced that a combined increase in temperature and salinity level can affect the germination rate of maize seeds negatively.

Temperature and water level combinations, on the other hand, showed that while there are no major differences in the germination rates between these treatments, 25°C proved to be the only one with a positive increase throughout the three (3) water levels used in the study. Individually, the output showed that as the level of salinity increases, the germination rate of maize seeds decreases. Meanwhile, it is the opposite for the temperature level, in a way that as it increases, the germination rate also increases. In relation, according to Sowiński et al. (2005), even if there is already an increase in temperature, seedling growth is still affected due to its inability to respond quickly to favorable environmental changes, which is in this case the change from low to moderate or high-temperature levels.

Temperature	Salinity	Water level (W)		T	* S		
°C (T)	level	6 ml	9 ml	12 ml			
	0	60.0	55.3	62.7	59	0.3	
20	1000	54.7	57.3	50.7	54	.2	
	1500	62.0	66.7	52.7	6	).4	
	0	50.7	56.7	54.0	53	3.8	
25	1000	50.0	46.7	51.3	49	0.3	
	1500	44.0	42.0	51.3	45	5.8	
	0	71.3	67.3	58.7	65	5.8	
30	1000	72.7	60.7	66.0	60	5.4	
	1500	59.3	54.7	70.0	61	.3	
LSD T*S*V	V		9.03		LSD T*S	5.21	
			T * W				
Temperature	e °C	6 ml 9 ml 12 ml			Mean Temp.		
20		58.9	59.8	55.3	58.	0 b	
25		48.2	48.4	52.2	49.	6 c	
30		67.8	60.9	64.9	64.5 a		
LSD T*W			5.21		LSD T	3.01	
			S * W				
Salinity level	ppm	6 ml	9 ml	12 ml	Mean	salinity	
0		60.7	<b>59.8</b>	58.4	59.	6 <mark>a</mark>	
1000	1000		54.9	56.0	56.7	ab	
1500		55.1	54.4	58.0	55.	9 <b>b</b>	
LSD s*w			5.21 <sup>N.S</sup>		LSD s	3.01	
			W				
Water leve	Water level		9 ml	12 ml			
Mean water l	evel	58.3	56.4	57.5			
LSD w			3.01 <sup>N.S</sup>				

Table 2. Overall Germination Rate of Maize Seeds.

#### 4.2. Seedling Length Analysis of Maize Seeds.

This chapter section presents the analysis of germinated maize seeds in terms of their seedling length, focusing on the rate of their growth across a 144-hour time duration and the correlation between the treatments included in the study.

Based on the table presented below (Table 3), it can be deduced that increasing the water level to germinate maize seeds provides a positive effect on maize seedling length while it is not the same for temperature and salinity. When the levels of temperature and salinity are increased, there will be a consequential decrease in the length of germinated maize seeds.

In terms of the interaction between salinity and temperature, high levels of the two negatively affect seedling length meanwhile low to medium levels of temperature and salinity are beneficial. The same cannot be said for the interaction between temperature and water levels, wherein a low-temperature level combined with a high level of water delivered a high seedling length for germinated maize seeds. In addition, when the water level is at 6ml while the temperature level is increasing from 20 to  $30^{\circ}$ C, the seedling length of maize seeds decreases.

In terms of salinity and water level interactions, results showed the same effect of temperature and water level interaction, that when the salinity level increase from 0ppm to 1500 level while maintaining the level of water in the treatment, the seedling length of germinated maize seeds are at its lowest. This suggests that water level is an effective buffer for the negative effect of high salinity levels on crop germination. According to Munns (2008), salinity negatively affects maize germination by increasing external osmotic potential, therefore, reducing water uptake during imbibition. That is why, it is necessary to have the optimum environmental conditions such as water, oxygen, and temperature for successful germination. Table 3. Seedling Length of Germinated Maize Seeds after 48 hrs.

Temperature	Salinity level	Wate	ater level (W)			T * S	
°C (T)	ppm (S)	6 ml	9 ml	12 ml	1 * 5		
	0	3.5	5.7	7.7		5.6	
20	1000	4.2	4.9	6.0		5.0	
	1500	3.1	6.1	4.8		4.7	
	0	5.7	6.4	6.5		6.2	
25	1000	6.5	7.0	5.1		6.2	
	1500	3.7	5.1	4.5		4.5	
20	0	5.1	4.8	5.0		5.0	
30	1000	5.3	4.6	5.2		5.0	
	1500	3.7	3.6	4.9		4.1	-
LSD T*S*	LSD T*S*W		1.02			ſ*S	0.59
		T * W	/		n		
Temperatu	re °C	6 ml	9 ml	12 ml	Mean Temp.		np.
20		3.6	5.6	6.2	5.1		
25		5.3	6.2	5.4	5.6		
		4.7	4.3	5.0	4.7		
LSD T*V	V		0.59		LSD T 0.34		.34
		S * W			1		
Salinity level	ppm	6 ml	9 ml	12 ml	Mea	Mean salinity	
0		4.8	5.7	6.4	5.6		
1000	5.3	5.5	5.5		5.4		
1500		3.5	4.9	4.8	4.4		
LSD s*w			0.59		LSD s	0	.34
		W	-	1			
Water level		6 ml	9 ml	12 ml			
Mean water		4.5	5.4	5.5			
LSD w			0.34				

The table below (Table 4) presents the seedling length of the germinated maize seeds after 72 hours. If compared to the 48-hour seedling length results, a significant increase can be noticed among all treatments. In relation, the temperature level of 30°C showed the best performance for the seedling length of germinated maize seeds while a median level of salinity at 1000ppm and a high level of water (12ml) posited the same results. This shows that at 72 hours of germination for maize seeds, higher temperature, and water levels are necessary while only an average level of salinity is the best for seedling length.

In terms of temperature and water interactions, the table shows that a difference in temperature levels (20°C and 30°C) can result in a difference in seedling length of germinated maize seeds, even with the same water level. Higher temperature levels resulted in higher seedling length, while it is the opposite of the germinated maize length for lower temperature levels (20°C). On the other hand, salinity and water level interactions showed that as the salinity level increases, the seedling length decreases as well even if the water level is maintained at 12ml. More so, low water level and high salinity level is not good treatment to get a better seedling length. The combination of the two aforementioned resulted in poor performance of germinated maize seeds in terms of seedling length.

Temperature	Salinity level	Water level (W)				T * S		
°C (T)	ppm (S)	6 ml	9 ml	12 ml				
	0	4.6	8.0	10.3		7.6		
20	1000	6.1	7.1	7.2		6.8		
	1500	4.2	7.3	6.3		5.9		
	0	7.8	7.8	8.3		8.0		
25	1000	8.5	9.0	7.3		8.3		
	1500	5.2	7.1	6.5		6.3		
• •	0	9.4	7.4	6.9		7.9		
30	1000	9.9	8.7	9.3		9.3		
	1500	7.8	5.0	10.1		7.6		
LSD	T*S*W	0.96			LS	D <sub>T*S</sub> 0.56		
			T * W					
Tempera	ature °C	6 ml	9 ml	12 ml	Ι	Mean Temp.		
2	0	5.0	7.5	7.9		6.8		
2	5	7.2	8.0	7.4		7.5		
3	0	9.0	7.0	8.8		8.3		
LSD	T*W		0.56		LSD	0.32		
			<u>S * W</u>					
Salinity le	evel ppm	6 ml	9 ml	12 ml	N	<u>Iean salinity</u>		
(	)	7.3	7.7	8.5		7.8		
10	00	8.2	8.3	7.9		8.1		
15	00	5.8	6.5	7.6		6.6		
LSD	LSD s*w		0.56		LSD	0.32		
	W							
Water	r level	6 ml	9 ml	12 ml				
Mean water level		7.1	7.5	8.0				
	D w		0.32					

Table 4. Seedling Length of Germinated Maize Seeds after 72 hrs.

Table 5 shows the results of the analysis of the seedling length of germinated maize seeds after 96 hours. From there, it can be deduced that the same trend was continued from the previous observation of the seedling length after 72 hours. High levels of temperature and water (30°C and 12ml) and a medium level of salinity at 1000ppm contributed to the longer seedling length of germinated maize seeds. In terms of temperature and salinity interactions, 30°C and 1000 ppm proved to be positively significant for the seedling length of germinated maize seeds while low temperature (20°C) and high salinity (1500 ppm) showed the opposite results.

In terms of water and salinity combinations, even if the seedling length is not uniformly increasing, 1000ppm is the best level of salinity to pair with increasing levels of water levels to get the best seedling length for germinated maize seeds. Relatedly, 12ml of water level is the ideal combination for the three levels of salinity treatments to get a high seedling length. In terms of temperature and water interactions, 6ml of water paired with 30°C of temperature showed the highest seedling length result of maize seeds. However, 12ml of water level and

30°C of temperature also showed almost similar results. From this, it can be deduced that at this point of germination, the water level is a significant factor for seedling growth.

Temperature	Salinity level	Wat	er level	(W)	T * S	
°C (T)	ppm (S)	6 ml	9 ml	12 ml		
•••	0	5.9	9.8	12.7	9.4	
20	1000	8.0	9.1	9.6	8.9	
	1500	5.3	9.0	7.4	7.2	
25	0	9.6	10.1	10.0	9.9	
25	1000	11.0	12.2	9.0	10.7	
	1500	6.3	9.7	8.2	8.1	
20	0	11.4	9.0	8.1	9.5	
30	1000	13.0	13.0	12.7	12.9	
	1500	12.8	7.3	15.5	11.9	
LSD	T*S*W		1.19		LSD <sub>T*S</sub> 0.69	
		T * W	7			
Tempera	ature °C	6 ml	9 ml	12 ml	Mean Temp.	
2	0	6.4	9.3	9.9	8.5	
2.	5	9.0	10.7	9.1	9.6	
3	0	12.4	9.7	12.1	11.4	
LSD	T*W		0.69		LSD т 0.40	
		S * W	7			
Salinity le	evel ppm	6 ml	9 ml	12 ml	Mean salinity	
		9.0	9.6	10.3	9.6	
10	00	10.7	11.4	10.4	10.9	
15	1500		8.7	10.4	9.1	
LSD s*w			0.69		LSD s 0.40	
		W				
Water level		6 ml	9 ml	12 ml		
Mean water level		9.3	9.9	10.4		
LSI	O w		0.40			

Table 5. Seedling Length of Germinated Maize Seeds after 96 hrs.

The table below (Table 6) presents the results of seedling length analysis of germinated maize seeds after 144 hours. Accordingly, median levels of water and salinity (9ml and 1000ppm) paired with high temperature at 30°C provided the highest seedling length of germinated maize seeds across all treatment combinations. Meanwhile, low water and temperature levels (6ml and 20°C) paired with high salinity (1500ppm) do not positively impact maize seedling length. In terms of temperature and salinity interactions, both 25°C and 30°C temperature levels have similarities with seedling length growth if the salinity level is both at 1000 ppm. Low temperature and high salinity level, on the other hand, is not a good combination for the growth of maize seedlings.

In terms of temperature and water level interactions, 6ml and 9ml of water level with a 30°C of temperature have two of the highest seedling length among any other treatments. Delving further, with the same temperature level, the seedling length of germinated maize seeds

decreases, however, as the water level increases. In terms of water and salinity interactions, higher seedling lengths are observed for a salinity level of 1000ppm even as the water level increases from 6ml to 12ml. It is also important to note that there is a decrease in the seedling length of germinated maize across varying water levels when the salinity level is increased. Table 6. Seedling Length of Germinated Maize Seeds after 144 hrs.

Temperature	Salinity level	Wa	ater level	(W)	T * 5	S
°C (T)	Ppm (S)	6 ml	9 ml	12 ml	-	
	0	7.6	16.0	13.5	12.4	
20	1000	9.4	12.2	17.4	13.0	
	1500	8.0	10.2	10.3	9.5	
	0	10.8	16.1	10.6	12.5	5
25	1000	17.1	14.5	17.7	16.4	
	1500	11.4	13.0	12.4	12.3	
	0	14.2	12.4	12.7	13.1	
30	1000	17.6	19.6	9.9	15.7	1
	1500	16.6	14.8	14.8	15.4	ļ
LSD 1	[*S*W		1.46		LSD T*S	0.84
		۲ ·	* W			
Tempera	ture °C	6 ml	9 ml	12 ml	Mean T	emp.
20		8.3	12.8	13.7	11.6	
25	5	13.1	14.5	13.6	13.7	1
30		16.1	15.6	12.5	14.7	1
LSD	T*W		0.84		LSD	0.49
		S *	* W			
Salinity le	vel ppm	6 ml	9 ml	12 ml	Mean sa	linity
0		10.9	14.8	12.3	12.7	
100	1000		15.5	15.0	15.1	
1500		12.0	12.7	12.5	12.4	
LSD s*w			0.84		LSD	0.49
W						
Water level		6 ml	9 ml	12 ml		
Mean wa	ter level	12.5	14.3	13.3		
LSE	W		0.49			

#### 4.3. Radicle Length Analysis of Maize Seeds.

In this section, the analysis of the radicle length of germinated maize seeds will be discussed. It will include the results of the individual treatments, as well as the interactions between the two treatments, and ultimately between the three included in the analysis (temperature, water, and salinity).

Table 7 shows the radicle length of germinated maize seeds after 48 hours. In terms of temperature, there is no difference between the radicle lengths of maize seeds at 20°C and 25°C. In addition, there is not much difference as well between 0ppm and 1000ppm of salinity levels and between 9ml and 12ml water levels. In terms of the interactions between temperature and

salinity, it can be observed that radicle length is high when the salinity level is at 0ppm even when the temperature level is increasing. In terms of temperature and water level interactions, low water level and low-temperature level together can negatively affect the radicle length of germinated maize seeds. In addition, increasing the temperature level while maintaining the water level can also lead to a decrease in the radicle length of maize seeds. This is supported by the findings of Khaeim et al. (2022) where accordingly, although both radicle and shoot is affected by increasing or decreasing temperature levels, one of them is more affected than the other.

For salinity and water interactions, 0ppm of salinity level is beneficial to the radicle growth of germinated maize seeds, if the water level is being increased. However, one can observe that as the salinity level is increased while maintaining water levels, there is a general decrease in the radicle length of germinated maize seeds, showing that salinity level is a critical factor in the germination and growth of maize.

Temperature	Salinity level	Wa	iter level	(W)		T * S
°C	ppm	6 ml 9 ml 12 ml				
	0	2.4	4.4	5.8		4.2
20	1000	3.1	3.2	4.4		3.6
	1500	2.4	4.7	3.6		3.6
	0	3.9	4.7	4.5		4.4
25	1000	3.7	5.1	3.2		4.0
	1500	2.8	3.8	2.7		3.1
	0	3.6	3.6	3.5		3.6
30	1000	4.0	3.3	3.8		3.7
	1500	2.0	2.8	3.2		2.6
LSD T*	S*W		0.94		LSD	0.54
		* T	• W			
Temperat	ure °C	6 ml	9 ml	12 ml	Μ	ean Temp.
20		2.6	4.1	4.6		3.8
25		3.5	4.5	3.5		3.8
30		3.2	3.2	3.5		3.3
LSD T	* <b>W</b>		0.54		LSD	0.31
		S *	W		-	
Salinity leve	el ppm	6 ml	9 ml	12 ml	Me	ean salinity
0		3.3	4.3	4.6		4.1
1000		3.6	3.9	3.8		3.8
1500		2.4	3.8	3.2		3.1
LSD s*w			0.54		LSD	0.31
W						
Water level		6 ml	9 ml	12 ml		
Mean wate		3.1	4.0	3.9		
LSD	W		0.31			

Table 7. Radicle Length of Germinated Maize Seeds after 48 hrs.

Presented in Table 8 are the results of the radicle length analysis of germinated maize seeds after 72 hours. Altogether, the combined treatment of 12ml water level, 0ppm salinity level, and 20°C temperature level had the highest increase in the radicle length of germinated maize seeds at 8.0cm. The lowest radicle length at 3.1cm came from the combined treatment of 6ml water level, 0ppm salinity level, and 20°C temperature level.

In terms of temperature and salinity interactions, 0ppm salinity level even with increasing temperature levels displayed higher radicle lengths compared to higher levels of salinity. However, at 30°C, the radicle length is higher when the salinity level is at 1000ppm, although there is not much significant difference when compared to the radicle length of maize seeds at 0ppm salinity level. In terms of temperature and weight combination, a high temperature (30°C) paired with a low water level (6ml) showed the highest radicle length

among other combinations. Coincidentally, the same water level (6ml) paired with the low temperature at 20°C had the shortest radicle length among other combinations.

In terms of the combination of water and salinity levels, it can be observed that generally, increasing salinity levels affect the radicle length of germinated maize seeds when the water level is constant. The results showed that as the salinity level increases but the water level is maintained, the radicle length gradually decreases.

Temperature	Salinity level	Water level (W)				T * S		
°C	ppm	6 ml	9 ml	12 ml				
	0	3.1	6.2	8.0		5.7		
20	1000	4.5	4.9	5.1		4.8		
	1500	3.3	5.3	4.8		4.5		
	0	5.6	5.5	5.9		5.7		
25	1000	4.7	6.6	4.5		5.3		
	1500	3.8	5.4	4.0		4.4		
	0	6.5	5.1	4.4		5.4		
30	1000	7.5	6.1	6.4		6.7		
	1500	5.2	3.5	6.7		5.1		
LSD 1	ſ*S*W		1.04		LSI	) <sub>T*S</sub>	0.60	
		Т	* W					
Tempera	ture °C	6 ml	9 ml	12 ml	Ν	Iean Tei	np.	
20	)	3.6	5.4	6.0		5.0		
25	5	4.7	5.8	4.8		5.1		
30		6.4	4.9	5.8		5.7		
LSD	T*W		0.60		LSD	0.	35	
		S	* W					
Salinity le	vel ppm	6 ml	9 ml	12 ml	Μ	lean sali	nity	
0		5.1	5.6	6.1		5.6		
100	)0	5.6	5.8	5.3		5.6		
150	00	4.1	4.7	5.2		4.6		
LSD <sub>S*W</sub>			0.60		LSD	0.	35	
			W					
Water		6 ml	9 ml	12 ml				
Mean wa	ter level	4.9	5.4	5.5				
LSE	) <sub>W</sub>		0.35					

Table 8. Radicle Length of Germinated Maize Seeds after 72 hrs.

In Table 9, the analysis of the radicle length of germinated maize seeds after 96 hours is presented. Individually, temperature and salinity don't have a significant difference when it comes to the average radicle length of germinated maize seeds. In terms of temperature and salinity, it is interesting to observe that when the temperature is at 20°C and 25°C, the radicle length decreases as the salinity level increases. However, it is not the same when the

temperature level is at 30°C, where the radicle length of germinated maize seeds is higher when the salinity level is higher as well (1000 ppm and 1500 ppm).

In terms of salinity and water level, as the water increases while maintaining the level of salinity at 0ppm and 1500ppm, the radicle length of germinated maize seeds increases. But, at 1000ppm with increasing water levels from 9ml to 12ml, the radicle length decreases. Finally, in terms of temperature and water level interactions, both 6ml and 12ml water levels resulted in high radicle lengths even when the temperature level with these treatments is at 30°C.

Combining the three, the results showed after 96 hours that the combined treatment of 12ml water level, 1500ppm salinity level, and 30°C temperature level had the highest radicle length at 10.1cm. The lowest, on the other hand, came from the combined treatment of 6ml water level, 0ppm salinity level, and 20°C temperature level. The poor growth of the radicle can be attributed to the loss of water in the petri dish already during this time.

Temperature	Salinity level	Water level (W)		Т	* S	
°C	ppm	6 ml	9 ml	12 ml	-	
	0	3.9	7.4	9.8	7	<b>'.0</b>
20	1000	6.0	6.3	6.9	6	5.4
	1500	3.9	6.2	5.4	5	5.2
	0	6.8	7.2	6.7	6	5.9
25	1000	6.1	8.5	5.4	6	5.7
	1500	4.6	7.3	5.0	5	5.7
	0	7.4	5.7	5.0		5.0
30	1000	9.7	8.7	8.2	8	3.9
	1500	8.9	4.9	10.1	8	3.0
LSD <sub>T*</sub>	S*W		0.99		LSD <sub>T*S</sub>	0.57
		Т	`* W			
Temperat	ure °C	6 ml	9 ml	12 ml	Mean	Temp.
20		4.6	6.6	7.4	6	5.2
25		5.8	7.7	5.7	6	5.4
30		8.7	6.5	7.8	7	<b>'.6</b>
LSD T	*W		0.57		LSD	0.33
		S	* W			
Salinity leve	el ppm	6 ml	9 ml	12 ml	Mean	salinity
0		6.0	6.8	7.2	6	5.6
1000		7.3	7.8	6.8	7	'.3
1500		5.8	6.2	6.8	6	5.3
LSD <sub>S*W</sub>			0.57		LSD	0.33
	W					
Water level		6 ml	9 ml	12 ml		
Mean wate	er level	6.4	6.9	6.9		
LSD	W		0.33			

Table 9. Radicle Length of Germinated Maize Seeds after 96 hrs.

Table 10 shows that after 144 hours, ten (10) combined treatments have reached an average radicle length of 10.0cm and above. The combined treatment of 9ml water level, 1000ppm salinity level, and 30°C temperature level had the highest recorded radicle length at 14.4 cm. On the contrary, the combined treatment of 12ml water level, 1000ppm salinity level, and 30°C temperature level had the lowest radicle length at 5.2cm.

Going further, interactions between temperature and salinity levels showed that 1000ppm is effective in maintaining the increase of radicle length of germinated maize seeds even with increasing temperature at 25°C to 30°C. In terms of temperature and water combinations, at increasing temperature levels, the radicle length increases even when the water level is constant at 6ml and 9ml. However, at 12ml paired with increasing temperature levels, the radicle length decreases already. Lastly, in terms of salinity and water level interactions, 1000ppm of salinity level is ideal for radicle length growth across the three different water levels used in the study, 6ml, 9ml, and 12ml). This is supported by the result of a study of Khalid et al. (2021) where it showed that there is a strong correlation between water level and radicle growth of germinated maize seeds.

Temperature	Salinity level	Water level (W)		(W)	T * S		
°C	ppm	6 ml	9 ml	12 ml			
20	0	5.9	13.1	9.8	9.6		
	1000	7.6	9.4	11.0	9.3		
	1500	6.1	6.8	7.2	6.7		
25	0	7.5	11.9	6.4	8.6		
	1000	13.7	10.3	13.0	12.3		
	1500	8.8	8.9	9.1	8.9		
30	0	8.9	9.0	7.9	8.6		
	1000	14.1	14.4	5.2	11.2		
	1500	12.3	9.5	10.8	10.8		
LSD <sub>T*S*W</sub>			1.04 LSD <sub>T*S</sub>		T*S	0.60	
T * W							
Temperature °C		6 ml	9 ml	12 ml	Mean Temp.		ոթ.
20		6.5	9.8	9.3	8.5		
25		10.0	10.4	9.5	10.0		
30		11.8	10.9	8.0	10.2		
LSD <sub>T*W</sub>			0.60		LSD 0.35		35
S * W							
Salinity level ppm		6 ml	9 ml	12 ml	Mean salinity		ity
0		7.4	11.3	8.1	8.9		
1000		11.8	11.4	9.7	11.0		
1500		9.0	8.4	9.0	8.8		
LSD <sub>S*W</sub>		0.60		LSD 0.35			
W							
Water level		6 ml	9 ml	12 ml			
Mean water level		9.4	10.4	8.9			
LSD w		0.35					

Table 10. Radicle Length of Germinated Maize Seeds after 144 hrs.

#### 4.4. Shoot Length Analysis of Germinated Maize.

Adjacent to measuring the radicle length of germinated maize seeds used in this study, presented in this section is the analysis of the shoot length of germinated maize seeds. It will include the results of the individual treatments, as well as the interactions between the two treatments, and ultimately between the three included in the analysis (temperature, water, and salinity) across four (4) time durations.

Table 11 shows the shoot length of germinated maize seeds after 48 hours. It can be noted that both the 25°C temperature level and 1000 ppm salinity level have the highest initial shoot length after 48 hours. On the other hand, an increase in the temperature and salinity levels (30°C and 1500 ppm) resulted in a decrease in the shoot length of germinated maize seeds. In terms of temperature and salinity interactions, high salinity levels paired with low temperature

proved to have a negative effect on the shoot length of germinated maize seeds. Specifically, in terms of high salinity stress, according to Carpýcý, Celýk, and Bayram (2009), the entry of sodium and chloride ions during the development of the seedling causes toxicity in the plant cells, and in turn, decreases the rate of germination together with the growth of the seeds that have already germinated

In terms of temperature and water combinations, a common occurrence from previous discussions, as the temperature level increases to 30°C while maintaining the water level, the shoot length decreases after achieving its highest length at 25°C. Salinity and water level interactions showed that at 1500ppm paired with lower water levels (6ml and 9ml), shoot length is at its lowest. Altogether, only one (1) treatment of combined water, salinity, and temperature levels, managed to have a shoot length of 2.0cm and above at 2.8cm for its germinated maize seeds (6ml water level, 1000ppm salinity level, and 25°C temperature level). On the other hand, the two combined treatments did not reach the 1.0cm threshold at 0.7 (6ml water level, 1500ppm salinity level, and 25°C temperature level.

Temperature	Salinity level	Water level (W)		T * S		
°C	ppm	6 ml	9 ml	12 ml		
	0	1.1	1.3	1.9	1.4	
20	1000	1.1	1.7	1.6	1	.5
	1500	0.7	1.3	1.2	1	.1
	0	1.8	1.7	2.0	1	.8
25	1000	2.8	1.9	1.9	2.2	
	1500	0.9	1.3	1.9	1	.4
• •	0	1.5	1.2	1.4	1	.4
30	1000	1.3	1.3	1.5	1	.4
	1500	1.8	0.8	1.7	1	.4
LSD T	*S*W		0.29		LSD T*	s <b>0.17</b>
		T * W	7			
Temperature °C		6 ml	9 ml	12 ml	Mean Temp.	
20		0.9	1.4	1.5	1.3	
25		1.8	1.6	1.9	1.8	
30		1.5	1.1	1.5	1.4	
LSD T*W		0.17		LSD 0.10		
S * W						
Salinity level ppm		6 ml	9 ml	12 ml		
0		1.4	1.4	1.8	1.5	
1000		1.7	1.6	1.6	1.7	
1500		1.1	1.1	1.6	1.3	
LSD s*w			0.17		LSD	0.10
W						
Water level		6 ml	9 ml	12 ml		
Mean water level		1.4	1.4	1.7		
LSD		0.10				

Table 11. Shoot Length of Germinated Maize Seeds after 48 hrs.

Table 12 presents the shoot length of germinated maize seeds after 72 hours. The results showed that in terms of temperature and salinity level interactions, there is a spike in the decrease of shoot length of maize seeds at 20°C and 25°C when the salinity level is increased from 1000 to 1500 ppm. In terms of temperature and water levels, 9ml of water level showed the least change in the growth of shoot length of germinated maize seeds while the other two (6ml and 12ml) contributed either increasing or decreasing spikes to the shoot length.

In terms of salinity and water level interactions, 1000ppm paired with the three levels of water (6ml, 9ml, and 12ml) showed the best shoot length of germinated maize seeds. This indicates that salinity level contributes not only to the shoot length but also to the eventual growth of maize seeds. Moreover, after 72 hours, the combined treatment of 6ml water level, 1000ppm salinity level, and 25°C temperature level recorded the highest shoot length for its

germinated maize seeds at 3.7cm. The lowest is the combined treatment of 6ml water level, 1500ppm salinity level, and 20°C temperature level at 1.0cm.

Temperature	Salinity level	Water level (W) T * S					
°C	ppm	6 ml	9 ml	12 ml			
	0	1.5	1.8	2.3	1.9		
20	1000	1.6	2.2	2.2		2.0	
	1500	1.0	2.1	1.4	1.5		
	0	2.2	2.2	2.3	2.3		
25	1000	3.7	2.5	2.7	3.0		
	1500	1.5	1.7	2.6		1.9	
	0	2.8	2.2	2.5	2.5		
30	1000	2.3	2.6	2.9	2.6		
	1500	2.7	1.5	3.4	2.5		
LSD T*S	LSD <sub>T*S*W</sub>		0.56			D <sub>T*S</sub>	0.32
		T *	W				
Temperature °C		6 ml	9 ml	12 ml	Mean Temp.		mp.
20		1.4	2.0	2.0	1.8		
25		2.5	2.1	2.5	2.4		
30		2.6	2.1	2.9	2.5		
LSD <sub>T*W</sub>		0.32			LSD	0	.19
S * W							
Salinity level ppm		6 ml	9 ml	12 ml	Mean salinity		inity
0		2.2	2.1	2.4	2.2		
1000		2.6	2.4	2.6	2.5		
1500		1.7	1.8	2.5	2.0		
LSD <sub>S*W</sub>		0.32			LSD	0	.19
W							
Water level		6 ml	9 ml	12 ml			
Mean water level		2.2	2.1	2.5			
LSD w			0.19				

Table 12. Shoot Length of Germinated Maize Seeds after 72 hrs.

In the table below (Table 13), the shoot length analysis of germinated maize seeds after 96 hours is presented. It can be seen that higher temperatures ( $30^{\circ}$ C) and higher water levels (12ml) are the best in the growth of germinated maize shoots, while mid-level salinity at 1000ppm is the best among other levels. According to a study by Khaeim et al (2022), the range of 20-30°C is ideal for maize seeds to initiate germination. Radicle emergence starts after 24-36 hours when the temperature level is at 28°C.

In terms of temperature and salinity interactions, low temperature at 20°C with increasing salinity levels contribute a negative effect to the shoot length of germinated maize seeds. On the other hand, in terms of water and temperature combinations, a gradual increase

in the temperature levels even while maintaining water levels holds a positive effect on the shoot length.

Moreover, the results showed that increasing the water level while increasing the salinity level at the same time helps maintain the growth of germinated maize shoots. This coincides with the previous results that the negative effect of high salinity levels can be buffered by increasing the water levels in the treatments.

Temperature	Salinity level	Water level (W)		T * S	3	
°C	nnm	6 ml	9 ml	12 ml		
	0	2.0	2.4	2.9	2.4	
20	1000	2.0	2.8	2.7	2.5	
	1500	1.4	2.8	2.0	2.1	
	0	2.8	3.0	3.3	3.0	
25	1000	5.0	3.6	3.6	4.1	
	1500	1.7	2.4	3.2	2.4	
	0	4.0	3.2	3.1	3.5	
30	1000	3.3	4.3	4.5	4.1	
	1500	3.9	2.3	5.4	3.9	
LSD T*	S*W		0.50		LSD <sub>T*S</sub> 0.29	
		T *	∗ W			
Temperature °C		6 ml	9 ml	12 ml	Mean Temp.	
20		1.8	2.7	2.5	2.3	
25		3.2	3.0	3.4	3.2	
30		3.7	3.3	4.4	3.8	
LSD <sub>T*W</sub>		0.29		LSD 0.17		
		S *	* W		· · ·	
Salinity level ppm		6 ml	9 ml	12 ml	Mean salinity	
0		2.9	2.9	3.1	3.0	
1000		3.4	3.6	3.6	3.5	
1500		2.3	2.5	3.5	2.8	
LSD S	*W		0.29		LSD 0.17	
W						
Water level		6 ml	9 ml	12 ml		
Mean water level		2.9	3.0	3.4		
LSD w			0.17		]	

Table 13. Shoot Length of Germinated Maize Seeds after 96 hrs.

Table 14 shows the results of the analysis of the shoot length of germinated maize seeds after 144 hours. When consolidated, the combined treatment of 12ml water level, 1000ppm salinity level, and 20°C temperature level have the highest shoot length of germinated maize seeds at 6.4cm. The lowest recorded is for the combined treatment of 6ml water level, 0ppm salinity level, and 20°C temperature level at 1.7cm. In terms of temperature and salinity interactions, high temperature at 30°C with increasing salinity level had the highest shoot length

of germinated maize seeds. On the contrary, 20°C of temperature has the lowest shoot length which can be interpreted as that temperature can significantly affect the growth of maize, even with or without other combinations of abiotic stresses.

In terms of temperature and water level interactions, the shoot length of germinated maize seeds continues to increase with temperature as long as the water level is maintained at 12ml. This coincides with the result where individually, 12ml contributed the highest shoot length among other water levels included in the study. In terms of salinity and water level combinations, while lower levels of salinity paired with increasing water levels resulted in high shoot length, 1500ppm which is the maximum salinity level in the study resulted in a decrease in the length of germinated maize shoots even after increasing water levels. This is supported by the study of El Sabagh et al. (2021) wherein accordingly, the length and severity of the salinity stress on maize can negatively affect the growth and yield of the crop, especially during the initial growth stage of the crop when maize is highly sensitive to the stress.

Temperature	Salinity level	Water level (W)			T * S		
°C	ppm (S)	6 ml	9 ml	12 ml	-		
	0	1.7	2.9	3.7		2.8	
20	1000	1.9	2.8	6.4		3.7	
	1500	1.9	3.4	3.2	2.8		
	0	3.3	4.2	4.2	3.9		
25	1000	3.3	4.2	4.7	4.1		
	1500	2.7	4.1	3.3		3.4	
	0	5.2	3.4	4.8	4.5		
30	1000	3.5	5.3	4.7	4.5		
	1500	4.3	5.3	4.1	4.6		
LSD <sub>T*</sub>	S*W		0.83		LSD <sub>T*S</sub> 0.48		0.48
		T ·	* W				
Temperature °C		6 ml	9 ml	12 ml	Mean Temp.		np.
20		1.8	3.0	4.4	.4 3.1		
25		3.1	4.2	4.1	3.8		
30		4.4	4.7	4.5	4.5		
LSD T*W		0.48		LSD 0.28			
S * W							
Salinity level ppm		6 ml	9 ml	12 ml	Mean salinity		nity
0		3.4	3.5	4.2	3.7		
1000		2.9	4.1	5.3	4.1		
1500		3.0	4.3	3.5	3.6		
LSD S	*W		0.48		LSD 0.28		28
W							
Water level		6 ml	9 ml	12 ml			
Mean water level		3.1	4.0	4.3			
LSD w			0.28				

Table 14. Shoot Length of Germinated Maize Seeds after 144 hrs.

### 5. CONCLUSION AND RECOMMENDATIONS

This section presents the conclusion drawn by the researcher based from the findings of the study. The recommendations for future research are also presented in this chapter.

### **5.1 Conclusions**

Based on the findings that were discussed earlier, the researcher was able to draw these conclusions:

- 1. The variations in the treatments that were used in the study provide significant effects on the germination rate of maize seeds, as well as on the seedling, radicle, and shoot length of the germinated seeds.
- 2. Fluctuations in germination rate across the period are caused by the combination of abiotic stresses such as water, salinity, and temperature. Hence, it is important to determine the appropriate combinations between the three, while considering the other external factors as well in field conditions.
- 3. The best-combined levels of water, salinity, and temperature for germination of maize seeds are 6ml water level, 0ppm salinity level, and 30°C temperature level. This is if all conditions are held constant. Upon closer inspection, the combination of 6ml water level and 30°C temperature level together with the variations in salinity level has a positive significant effect on the germination rate of maize seeds.
- 4. The combined treatment of 9ml water level, 1500ppm salinity level, and 25°C temperature level registered the lowest germination rate for maize seeds. It can be concluded that increasing water level while decreasing temperature level with high salinity level negatively affects the germination rate of maize seeds.
- 5. Finally, ideal combinations of temperature, water, and salinity levels can guarantee the high viability of maize seeds, however, this viability is not necessarily an indicator of high growth and development.

## 5.1. Recommendations

- Further variability in the treatments (temperature, water, and salinity) can be explored. The results from these studies will in turn provide a wider range of perspectives to those areas around the world with the same environmental conditions.
- 2. Since this study was conducted in the laboratory where the parameters are controlled by specific levels, it can be also possible to conduct field-based

experiments on the effect of abiotic stresses on maize germination. This can provide an anchored result to the setting in which the study was conducted.

#### 6. SUMMARY

Maize is a major crop grown around the world for food and forage. As adaptive as they are to a wide range of soil and climatic conditions, they are also vulnerable to the effects of climate change that agriculture is currently facing these days. These effects are usually in the form of environmental stresses such as drought, high temperature, waterlogging, and high salinity levels in the soil. These stresses, in turn, are the major constraints that limit maize production year after year worldwide.

Relatedly, it is a reality that environmental stresses commonly affect agricultural regions in combinations at once, such as drought and high salinity, high temperature, and salinity, or waterlogging and salinity. That is why, this study is conducted to determine the significance of the effect of combined abiotic stresses on maize germination because the reality in the field is that there is not only one individual abiotic stress present to affect, there are usually two or more. This study may provide additional information on finding solutions to mitigating the effects of climate change on crop production.

The experiment for the study was conducted in the laboratory of the Institute of Agronomy at the Hungarian University of Agriculture and Life Sciences (MATE) in Gödöllő, Hungary. Seed samples of horse-tooth maize were used and tested for their germination rate, seedling, radicle, and shoot growth under combined abiotic stress conditions. The experiment had three replications with twenty-seven (27) treatments in a Memmer-type climatic chamber. These treatments were then observed for a period of seven (7) days, excluding weekends since the laboratory is closed. The data gathered was then analyzed through mean and one-way ANOVA.

The results of the study showed that in general, the three variables that were included (temperature, water, and salinity) have a significant effect on the germination of maize seeds, either positively or negatively. High temperature, water, and salinity levels when combined have adverse effects on the germination rate as well as the length of radicle and shoot of maize seeds. However, the same can be said as well for low temperature, water, and salinity levels. A combination of average levels of these treatments is the best for maize viability and development.

In addition, high temperature and high salinity proved to be a negative combination to the seedling length of germinated seeds. The lower the temperature level as well as the lower the salt concentration, the better it is for maize. Temperature and water interactions also showed that when either one is increased, there will be a significant difference in the overall radicle length of germinated maize seeds. Moreover, interactions between water and salinity revealed

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that a higher water supply for maize germination can buffer the negative effect of salinity. Finally, it can be derived from the results that a high germination rate of maize seeds can be achieved by the combinations of the three treatments, however, high growth and development are not necessarily assured. Certain combinations can bring forth a low seedling rate as well as low radicle and shoot length of the germinated maize seeds.

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Signed below, Michael Dela Rosa, student of the Szent István Campus of the Hungarian University of Agriculture and Life Science, at the MSc Course in Crop Production Engineering declares that the present Thesis is my work and I have used the cited and quoted literature in accordance with the relevant legal and ethical rules. I understand that the one-page summary of my thesis will be uploaded on the website of the Campus/Institute/Course and my Thesis will be available at the Host Department/Institute and in the repository of the University in accordance with the relevant legal and ethical rules.

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