Effects of VPD and Salinity on the Leaf area development and Transpiration in two contrasting genotypes of Sweet potato.



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List of Abbreviations:

ABA Abscisic acid Ca²⁺ Calcium Cl⁻ Chlorine CO_2 Carbon dioxide **CWL Cumulative Water Loss** DAP Days After Planting **Actual Vapour Pressure** Ea Saturated Vapour Pressure Es(T) EC **Electrical Conductivity** Grams g Kilograms Kg LA Leaf Area Mg^{2+} Magnesium MJ Mega Joules m Mol Milli Mole Mt Million ton Ν Nitrogen Na⁺ Sodium NaCl Sodium Chloride NO_{3-} Nitrate O_2 Oxygen Phosphorus PAR **Plant Active Radiation** Potential of Hydrogen рΗ

K Potassium

kPa Kilo Pascals

rH Relative Humidity

SOD Super Oxide Dismutase

USA United States of America

VP sat Saturated Vapour Pressure

VP air Vapour Pressure in Air

VPD Vapour Pressure Deficit

WL Water Loss

1. Introduction:

Sweet Potato is the sixth most important food crop in the world (Lebot, 2010). Worldwide, about 105 million metric tons are produced annually. Most of the production comes from developing countries. It is a starch crop with high nutritional values. It is a rich source of beta carotene, vitamin B, C and E. It is used for human consumption as well as animal feed (Fuglie, 2007). Cattle fed with sweet potato vines emit reduced amounts of methane. An increase in population and rapid industrialization has a greater effect on global food production. Climate change is also a major challenge in the present situation to meet the food requirements of the global population. To meet all these needs, an ecofriendly industrial crop is needed. The sweet potato as a nutritional rich crop can meet the food and nutritional security challenge and encourage sustainability in the coming year (Motsa et al., 2015). Recent studies stated that sweet potato has a lot of medicinal values and can be used against cancer, diabetes, and inflammatory problems (Kwak, 2019).

Salinity is one of the most important problems due to its adverse effect on agriculture and sustainability (Haque, 2006). Both Natural and human-induced actions can cause salinity problems in all kinds of climatic conditions (Mahajan & Tuteja, 2005). Mostly, salinity is the main problem in arid and semi-arid areas where salts come out of the root zone due to reduced water availability for the plants (Kaya et al., 2003). Salinization is increasing every day and spreading to all countries. In the future, there will be no country in the world without this salinization problem. This salinization may lead to an increase in climate change in the future. The rise in temperature due to climate change can results in an increase in evaporation thereby causing salinization. It ultimately reduces the potential of ecosystem services. According to several reports, the increase in salinity and its expansion exceeds the area that was restored by reclamation and rehabilitation. Globally, 23% of the cultivated land is saline and 37% is sodic land (Shahid et al., 2018). From irrigated land, 25-30% are salinity effected and commercially unproductive (Rengasamy, 2006). Poor irrigation management and low-quality ground water particularly in arid and semi-arid regions are the main reasons for salinization (Metternicht & Zinck, 2003). In these areas, rainfall exceeds evapotranspiration which is another important reason for salinization. According to some reports, nearly ten million hectares per year of cultivated land are lost to salinity (Shahid et al., 2018). Salinity results in reduced plant growth due to Na+ toxicity and reduced uptake of essential nutrients such as calcium or potassium (Ruiz et al., 1997). Under high salt concentrations, the plant undergoes osmotic and ionic stresses. This leads to a reduction in leaf expansion rate and closure of stomata (Machado & Serralheiro, 2017). Salt stress may also lead to water stress which ultimately affects crop yield (Ozturk et al., 2004). Plant responses to saline

conditions vary among crop species. There is a chance of improving the potential of plants for salt tolerance through selection and breeding (Saddiq et al., 2021).

Vapour Pressure Deficit (VPD) is a function of air humidity and temperature and describes the drying power of the air. An increase in the VPD results in dry conditions and a decrease in VPD results in wet conditions. Transpiration in plants is influenced by VPD. As water is transpired by the leaves, roots take up water along with nutrients and the transpirational volume flow allows for apical transport. (Turner et al., 1984). Under high VPD, the plants transpire more due to the prevailing dry conditions in the atmosphere (Lösch, 1979). This leads to higher uptake of water and nutrients by the root system and may cause toxicity. In this situation, as part of the plant defence mechanism, the stomata close (Turner et al., 1984) which leads to reduction of CO₂ uptake and ultimately reduce plant growth (Lange et al., 1971). Under low VPD, the plant transpires less water than required which leads to reduced water and nutrient uptake. (McAdam & Brodribb, 2015). So, it is very important to maintain the optimum VPD conditions in the plants subjected to 0 mmol NaCl-led environmental conditions for the better growth of the plants. The optimum VPD changes from crop to crop. VPD depends on the temperature and air humidity. So, the manipulation in the VPD can be done by changing the air humidity and temperature (Merilo et al., 2018).

Though Sweet potato is moderately tolerant to salinity (Begum et al., 2015), where 50% of yield can be reduced with a salt concentration above 6 mS cm⁻¹ (O'Sullivan et al., 1997). There was very little information about the effects of salinity in the sweet potato. This study focuses on the combined effects of salinity and VPD on leaf area development and transpiration in two sweet potato varieties contrasting in salt tolerance.

1.1 Research Objectives and Hypothesis:

The main aim of the experiment was to know the effect of salinity and VPD on the leaf area development and transpiration in two contrasting sweet potato varieties.

The main objectives of the experiment were,

- 1. To study the effect of the salinity and VPD on the leaf area development and transpiration in sweet potato.
- 2. To study the genotypical effect on leaf area and transpiration under salt stress.
- 3. To understand the effect of salinity and VPD on sweet potato plant dry weight.

The following hypothesis was formulated from the above objectives:

- Salinity and Vapour Pressure Deficit affect the leaf area development in sweet potato genotypes.
- 2. Under different VPD levels, the salinity can influence the transpiration of sweet potato genotypes.

2. State of the art:

2.1 Salinity and Sources:

Salinity refers to the presence of electrolytic mineral solutes in the soil and water in higher amounts that are dangerous to plants. It is one of the major stresses and affects agricultural production mainly in the arid and semi-arid regions (de Oliveira et al., 2013). Increased salinization may result in loss of arable land. There were some reports that the loss of arable land due to salinization will be 50% by the middle of the 21st century (Mahajan & Tuteja, 2005). Salt can inhibit plant growth more than any other toxic substance in the soil. There are mainly two sources for this soil salinization. The primary salinization is from seashore salty marshes (Zhu, 2007). The secondary salinization is due to ineffective drainage systems in irrigated soils, irrigation with salt-rich water, land clearing, and high application of nitrogen and potassium salt-rich fertilizers (Gorji et al., 2015). Secondary salinization is a very serious source because most of the productive agricultural land is vanishing due to this reason. Poor drainage leads to evaporation of irrigation water, leaving the salts in the soil (Zhu, 2007).

2.1.1 Problems of salinity:

The problems caused by salt stress are mainly two ways. The first way is the toxicity of the salt ions to the plant cells. Sodium chloride has many salts. Both sodium and chloride ions can affect plant growth. The second way is due to osmotic stress or water deficit. The high salt concentration in the soil solution leads to a reduction of the osmotic potential which results in osmotic stress (Zhu, 2007). This osmotic

stress can inhibit cell expansion using abscisic acid. The sodium ions can also affect the uptake of potassium by the plant roots. This is due to the similarity in the chemical nature of both sodium and potassium. This leads to potassium deficiency in the plant (Zhu, 2007). Under salt stress, the Na⁺ and Cl⁻ compete with the other nutrients like K⁺, Ca²⁺, and NO³⁻. This competition results in a nutrient imbalance in the plant. There was a finding in the fennel plants that an increase in the concentration of NaCl results in an increase of Na and Cl and a decrease of N, P, K, and Mg levels (Jouyban, 2012). The N uptake was reduced with salt stress in the green bean was reported (Pessarakli, 1999). Prolonged salt stress may lead to physiological drought. The salt stress causes low water potential in the soil. Then, the plants feel difficulty in the uptake of the water and nutrients. This results in the water stress condition in the plant (Mahajan & Tuteja, 2005).

The main symptoms caused by salt stress are reduced growth, senescence, accelerated development, and death of the plant if the exposure period is very long. Out of these symptoms, the reduction of the growth is the major symptom that leads to the other symptoms. Sometimes, severe salinity shock results in programmed cell death. The salt stress also leads to the closure of stomata which results in the reduction of photosynthesis and causes photoinhibition and oxidative stress. The stomata closure is due to the synthesis of abscisic acid which can be induced by salt stress. The stomata close after the abscisic acid is transported to the guard cells (Zhu, 2007).

2.1.2 Remedies:

Crop production in salt-affected soils can be improved by removing excess salts from the root zone. There are different methods used for the removal of excess salts, such as flushing, scraping, and leaching. But these methods are very expensive. There is one more method that is relatively cheap and can be used by all types of farmers. That is the use of Halophytes. Halophytes are plants that can grow even in higher saline conditions and can remove the excess salts from the root zone. Some salttolerant grasses can be cultivated in the saline or saline-sodic soils to remove the fixed CaCo₃ in the soil through root action (Pessarakli, 1999). The other method for reducing the effect of salinity on the plants is a foliar application of nutrients. This can increase the salt tolerance of the plants by decreasing the Na⁺ and Cl⁻ injuries to the plants (Jouyban, 2012). Under salt stress, increased calcium supply can reduce the effect of sodium ions on the plant. The calcium can inhibit the sodium uptake mediated by non-selective cation channels. In sodium-challenged plants, the calcium can maintain the transport of potassium and sodium/potassium selectivity (Zhu, 2007). Salt exclusion or salt inclusion are two main strategies that help higher plants in tolerating the high salinity levels. The salt excluders can exclude salt from the whole plant or certain organs of the plant. These plants possess low Na⁺ and Cl⁻ ions because the membrane selectivity likes to take K⁺ over Na⁺. The salt accumulators use two strategies to survive even under high salt concentrations. The first one is resistance of cell membranes

to the high levels of intracellular salts, commonly seen in halophytes. The second one is taking off the excess salt accumulating into the plant. This results in the escaping of salt injurious effect (Jouyban, 2012).

2.2 Vapour Pressure Deficit:

Vapour pressure deficit is the difference between the amount of moisture in the air and the amount of moisture that air can hold when it gets saturated at a given temperature. The water holding capacity of the air reaches the maximum under saturated conditions. The moisture added after the maximum water holding capacity of the air condenses and results in liquid water deposition in the system. The amount of water present as a gaseous form in the air is known as Vapour pressure (VP air). Higher the vapor content higher the vapour pressure. The Vapour pressure when there is maximum water vapor in the air is known as Saturated Vapour Pressure (VP sat). Mathematically, VPD = VP sat-VP air (Prenger & Ling, 2000). VPD is useful for monitoring the condensation potential, disease threat and water requirement for the crops grown in the controlled conditions. The transpiration of the plants increases with increasing VPD levels, this may result in the addition of more moisture into the air. Under lower VPD conditions, the condensation of moisture in the air takes place and may lead to disease development in the plants. The canopy temperature used for the calculation of VPD gives clear information about how close the canopy is to the dew point (Prenger & Ling, 2000).

2.2.1 Plant responses to VPD:

Generally, the plant transpiration depends on the stomatal opening and closing which are mainly operated by the VPD. An increase in VPD & Temperature and a decrease in relative humidity may result in warm climate conditions. A plant can experience physiological stress due to higher water loss under higher VPD conditions, mostly more than 2 kPa. The stomata closure takes place under such conditions to avoid water loss. This may lead to the reduction of the CO₂ diffusion, thereby photosynthetic activity and ultimately the plant development. Apart from the disease threat problem due to condensation under low VPD, there is an increase in photosynthetic carbon due to the stomatal opening. But the relative humidity increases which may lead to the reduction of evapotranspiration. This can affect the flow of water and nutrients (Amitrano et al., 2019). There is a correlation between the VPD and ABA hormone which involves the developmental processes and stress response. The ABA accumulation increases with increasing VPD and vice versa. There were several reports that the ABA induce the stomatal closure under high VPD conditions. There are different short- and long-term mechanisms to decrease the water loss by higher VPD. The short-term mechanisms include several physiological and biochemical adjustments. These can help the plants within seconds to minutes. The

long-term mechanisms include modification of the structure of the organs during development depending on the plant plasticity (Amitrano et al., 2019).

2.2.2 Effect of VPD on plant growth, yield, and product quality:

Several findings say the changes in the VPD level can change the plant growth rate, biomass allocation, and plant development. The lower VPD level has a more positive effect on plant growth than the higher VPD. Thinner leaves with the larger area were observed in Rosa hybrida under low VPD conditions. An increase in the above-ground biomass, stem diameter, leaf length, and improved fruit development was observed in tomato plants under low VPD levels (Amitrano et al., 2019). According to Lu et al., 2015, tomato plantlets have higher plant growth in the lower VPD conditions compared to the higher VPD conditions (Lu et al., 2015). Higher VPD conditions combined with higher temperature and radiation can affect the quality attributes in the horticultural crops. These conditions can cause a reduction in the sugars, mineral composition, and carotenoids in fruits. This deterioration of fruit quality is because of the fewer chlorophylls which result in the reduction of the photosynthetic activity and migration of photosynthates to the sinks. The sugar content in the fruits was improved under low VPD conditions (Amitrano et al., 2019). Higher VPD can affect the supply of water to the fruit and increase the transpiration of fruit. The fresh weight of the tomato fruit was reduced under a high VPD level but there was no effect on the dry weight. The loss of water content from the fruit is the main reason for the reduction of the fresh weight under higher VPD levels. Physiological disorders of calcium were observed in the leaves of tomato plants under low VPD conditions. But no symptoms on the fruits were observed. There was also a finding that the fruits grown under low VPD conditions have shorter shelf life due to their rapid nature to become soft (Leonardi et al., 2000). In addition to the direct effect of VPD on the plant physiological stress, the higher VPD increases the water loss from the moist soils. This results in the drying of certain land surfaces which leads to more drought events (Grossiord et al., 2020).

2.3 Importance of leaf development:

Leaves are the important part of the plant that develops from the apical meristem and then becomes a complex organ with cell expansion and division (Tsukaya, 2013). The leaf area mainly depends on the cell number and cell size (Hu et al., 2020). The leaves are responsible for photosynthesis, respiration, and photo-perception (Alm et al., 1988). The leaf also helps in recognizing the duration, quality, and quantity of the light which helps in the plant development (Christensen & Weigel, 1998). The whole plant development mainly depends on the leaf development. Though two plants are similar in their genetic nature, leaves may differ between them. Leaf expansion and duration can affect crop growth and yield. Different parameters can affect the shape or size of the leaf. The leaf expansion decreases under low light intensity. Scarcity of water and other resources can also reduce the leaf size.

Salinity causes a significant reduction of leaf area in different crops (Bar & Ori, 2014). The parameters like leaf area, leaf number and leaf age mainly influence the photosynthesis. Among these, leaf area is the major factor in the plant growth. The leaf age and leaf area have positive correlation with the plant growth till the leaves developed fully (Jo & Shin, 2020). The plants with higher leaf area can have more photosynthetic area thereby high photosynthesis. This results in the higher development of the plant and higher yield. Both biotic and abiotic stresses can negatively affect the leaf development. This cause ultimate reduction of the yield of the crop. There were reports that increase in leaf area increased the yield of cotton crop (Jafri & Ahmad, 1995).

The leaf area management plays an important role in the plant yield in several crops. Increasing the intensity of light passing through the canopy by controlling the leaf area can increase photosynthesis. The leaf area can be controlled by the defoliation method. The defoliation of old leaves can give young leaves which helps in more photosynthesis, nutrient uptake, and crop productivity. In Solanaceae family plants, the change in the microclimate by adjusting the leaf area can result in higher product quality (Jo & Shin, 2020).

2.4 Transpiration:

Transpiration is the evaporation of water through different parts of the plant. The water loss through transpiration mainly occurs through the stomata. It also occurs through cuticle which accounts for 10% of total transpiration in the plant. The plant stem also losses water through the lenticels (Candia & Michaelian, 2010). Nearly 90% of moisture in the atmosphere comes from the water bodies, remaining 10% of the moisture is coming from transpiration. Stomatal transpiration involves mainly three mechanisms. They are a) The water present in the leaf moves due to osmotic diffusion to the intercellular space on the stomata through mesophyll cells, b) stomatal behaviour, mainly opening and closing, and c) movement of water vapor into the atmosphere from the intercellular spaces though stomata.

Transpiration depends on several external and internal factors. External factors are temperature, humidity, light, soil moisture availability, plant type, CO₂, and wind. As the temperature rises and relative humidity decreases, the plants transpire more, and this helps in the increase of relative humidity of the air. Then the transpiration decreases. This works as a continuous cycle in the plant system. The water status of the plants shows higher in the morning and night-time of the day than in the daytime. This is due to the higher transpiration in the daytime because of the higher light. It causes an imbalance between the amount of water received by the roots and transpired by the plant. The relative growth of the tomato plants was higher in the morning and night than in the daytime (Díaz-Pérez, 2018). The transpiration in the soils with less moisture availability is low. The plants dry up and cannot transpire due to a lack of moisture in the soil. The amount of water transpires by the plants

differs from crop to crop. An Oak tree loses 151,000 litres of water per year through transpiration. An acre maize crop loss 11,400 - 15,100 litres per day. Generally, the amount of water transpired by the leaf is more than its weight. The higher concentrations of CO_2 result in the closure of stomata which reduces the transpiration. Wind movement can affect transpiration. The warm air brings by the wind movement replaces the cool air around the plant and increases the transpiration of the plant. The internal factors are the water status of the plants and structural features of the plant. Reduction in the internal water status of the plants results in the reduction of transpiration. The structural features like surface area exposed to light, leaf angle, size, and several stomata influence the amount of water loss through transpiration.

Transpiration has both harmful and beneficial effects on the plant. Different findings reported that the harmful effects of transpiration were more than the beneficial effects (Clements, 1934). The plant can grow under 100% rH conditions where the transpiration is negligible. The plants need carbon for photosynthesis. This carbon is taken by the opening of stomata. This results in the evaporation of water from the leaves through the stomata. This water loss leads to dehydration and ultimately death of the plant (Candia & Michaelian, 2010). The transpiration affects the produce after harvest. It is a very big problem in horticultural crops. The fruits after harvest transpire and lose water through the surface areas. This water loss cannot be replaced. This leads to the deterioration of the quality of the product and gives less value in the market (Díaz-Pérez, 2018). There were also reports that transpiration could help the plants in different ways. It plays an important role in the reduction of the leaf temperature. The radiant energy observed by the leaf transforms into heat vaporized water and it reduces the leaf temperature. The air temperature was rarely exceeded more than 5°C in several plants, in which the transpiration is high. Transpiration plays an important role in the movement of the water and nutrients from the roots to the leaves. Once the nutrients enter the plants, they distribute to different locations. This results in the more rapid growth of the plant. The transpiration also helps in maintaining the turgidity of the cells. Transpiration results in the water movement from roots to the above parts of the plants through the xylem, the water keeps the cells turgid and avoids wilting of the plant (Clements, 1934).

2.5 Sweet Potato Crop:

Sweet Potato is a perennial plant of the Convolvulaceae originated from tropical America. Later it was taken to the South Pacific, Africa, Asia, and western pacific. It is grown in the tropical and sub-tropical regions of the world. It is a nutritious staple food crop. The plant has long veins. The leaves are lobed. The edible part is the tuber which is formed by the thickening of the feeder roots. There are mainly two cultivars grown generally. The staple varieties are under cultivation in the tropics. These are

mainly white and red. The other orange-fleshed varieties which have high sugar and less dry matter are cultivated in the USA. Here it is used as supplementary food (O'Sullivan et al., 1997).

Sweet potato is the third-largest root crop produced in the world. The Irish potato and cassava are in the first two positions with the annual production of 281 Mt and 164 Mt respectively. The annual production of sweet potato is 122 Mt. Highest production is from China with 54% of global production. Then Vietnam and Indonesia combinedly account for 4% of global production. The people of highland east Africa and the Melanesian Pacific consume sweet potato as a staple food sin their diet. In these countries, sweet potato is very important on a per capita basis (O'Sullivan et al., 1997). Sweet potatoes can grow in a wide range of environments. It can grow from humid tropics to mild temperate zones and from sea level to 2700 m altitude. It can also grow in semi-arid conditions. It can tolerate low temperatures at high altitudes. Due to the sensitivity towards water logging, it is cultivated on ridges or mounds (O'Sullivan et al., 1997).

Sweet potato has a high edible proportion, and it is short duration crop, where the crop is harvested in 140 days. It has a very high edible energy yield than other staple crops (O'Sullivan et al., 1997). Sweet potato can be boiled and consumed as a sole or mixed with other cereals, millets, or pulses. The flour of sweet potato is used as a sweetener for some foods in the semi-arid regions. In humid south regions, chips are made using sweet potatoes. In Nigeria, there are non-sweet cultivars of sweet potato due to their undesirability towards sweet cultivars. The damaged, small-sized tubers, leaves, and vines are used as feed for rabbits, sheep, and cattle (Ojeniyi & Abu, 2003). Generally, Root crops are weaker than cereals in the nutritional aspects. But the protein concentration of sweet potato is similar to that of rice. The protein content may vary based on different factors. It may improve by genetic or management practices. The average protein content of sweet potato in South pacific crops ranges from 0.46 to 2.93%. It is equal to 1.0 to 6.1 g/MJ. Sweet potato has high calcium, Vitamin C, and carotene (provitamin A). The carotene content is very high in the yellow to orange-fleshed cultivars. The people in the countries where rice is a staple food suffer mostly from Vitamin A deficiency. This results in blindness in children. Infant mortality also occurs under less acute deficiency conditions. In most Asian countries, these yellow-fleshed varieties are gathering importance. The shoot is consumed as a green vegetable in some Asian countries.

It is rich in protein, thiamine, carotene, riboflavin, folic acid, and ascorbic acid (O'Sullivan et al., 1997). The yield of sweet potato under a high input production system is 30-50 tons per hectare. But the potential yield of the sweet potato is 80-100 t/ha. This is not possible under practical conditions. The average yield under semi-subsistence economies is only 4-6 t/ha. The yield gaps indicate the potential for increasing food production. Reduction of land resources and interest in cash crops are mainly

decreasing the area under sweet potato cultivation in most of the African, Pacific, and Central American countries (O'Sullivan et al., 1997).

2.5.1 Source and sink:

Source and sink are two main aspects of any crop which decide the yield of the crop. Source represents the places where the assimilates are produced and sink represents where these produced assimilates are stored in the plant. in sweet potato, the leaves are the main part of the source, and the tubers represent the sink. The shoot of the plant also acts as the source in the early period of growth. There are two more terms called Source potential and Sink capacity. The leaf area and photosynthetic rate comes under source potential and the number of tubers and mean tuber weight are considered sink capacity. There is wide variation between these two in the sweet potato crop. The yield may limit by either of these two. It is improper to decide which has a more limiting effect on the yield. There were different studies made to understand the source potential and sink capacity in the sweet potato. Most of the studies reported that both source potential and sink capacity have equal contributions in deciding the yield of the crop. But the period of contribution varies in the duration of the crop. The sink capacity has less limiting than source potential in the early growth period. But after the formation of tubers, both Source potential and sink capacity has equal contribution in deciding the yield. Previous findings have reported that there was a positive correlation between the shoot weight and tuber weight. There were also contrasting findings of the above statement which stated a negative correlation between the shoot weight and tuber weight. The shoots after a certain level of growth use most of the photosynthates and this leads to small tubers. The high-yielding varieties with high sink capacity were grafted on low-yield varieties. This increases the source potential in the low-yielding varieties. The source should not be hyperactive. It may result in less tuber yield. So, the most desirable combination is an active source with high sink capacity (Ravi & Saravanan, 2012).

2.6 Effect of Salinity and Humidity on different plant parameters:

Sweet potato has shown moderate tolerance to salinity. The cellular K⁺/Na⁺ level was maintained above 1 at 8 dSm⁻¹. The yield of sweet potato was decreased by 50% under the salinity level of above 8 dSm⁻¹ in laboratory conditions. The same kind of results was obtained under field conditions also. The growth of the plant gets reduced under salt stress. This is due to the higher energy requirement of the plants under salt stress. In the wheat crop, there was a report that states 3% to 4% additional energy was necessary under salt stress conditions. The growth of the seedling was affected due to the less water uptake by the seed because of the osmotic stress under saline conditions. The leaf number in tomato cultivars was decreased from 33.33% to 48.39% at 0.5% NaCl and 34.33% to 84.62% at 1.0% NaCl. The root is the main plant part which is exposed first to the salt. The root length and number were reduced 24.88 to 70.40% and 43.48 to 81.82% respectively at 1.0% NaCl. Super Oxide Dismutase

(SOD) activity in plants was increased under salt stress conditions. The increased level is high in salt-tolerant genotypes than sensitive genotypes. High SOD activity in salt-tolerant cultivars was observed in pea, wheat, and rice crop (Dasgupta et al., 2008). In sweet Potato, the plants grown under 85% RH produced an extra storage root per plant than the plants grown under 50% RH conditions. The fresh and dry weight of the storage root was 29% and 25% higher, respectively under 85% RH than 50% RH. The fresh and dry weight of the foliage was higher at 50% RH than 85% RH. The growth rate was higher in the plants grown under 85% RH than 50% RH. Edible biomass was also higher under 85% RH conditions. The same results were obtained under field conditions also. The leaf temperature was lower under 85% RH conditions. So, ultimately the sweet potato has shown a higher growth rate, storage root yield, and edible biomass under higher RH conditions. This is due to the higher stomatal conductance or higher cell enlargement or combination of both. Under higher RH conditions, the photosynthates were allocated to the storage tissues than foliage (Mortley et al., 1994).

2.7 Hydroponic System:

Dr. Gericke, a professor from California has used the word "Hydroponics" for the first time in 1929. It was derived from two Greek words, hydro means water, and ponos means labor. Hydroponics is defined as the process of growing plants in liquid nutrient solution with or without the use of artificial media. There are different mediums used in a hydroponic system. The most popular mediums are the clay, coir, perlite, vermiculite, and wood fiber. This system is mainly used for vegetable and ornamental plants cultivation. During World War II, hydroponic systems were used for food production to the troops (Dunn, 2010).

Hydroponic systems are mainly classified as open and closed systems. Open systems are also called as run to waste systems. In these systems, the nutrient solution is used only once, and it is not reused. This helps in reducing the risk of the spread of infections and no need for nutrient solution maintenance. But the wastage of huge amounts of water and nutrients is the primary disadvantage of this system. Closed systems are the systems where the nutrient solution can be replenished and reused. Monitoring and maintenance of the nutrient ratios in the solution are very important. The addition of water to raise the volume of nutrient solution and the addition of stock solution to increase the concentration of nutrient solution are the two main elements in the maintenance of the nutrient solution. This system saves a lot of water and nutrients. The closed systems use 20-40% less water than open systems. But, monitoring and maintenance is the most difficult work. Ultimately, the nutrient solution in the closed systems can be changed after a week or two weeks. But, the system with proper monitoring and maintenance with no change of the nutrient solution is the most desirable hydroponic system (Christie, 2014).

There are different pros and cons to the hydroponic system. The pros have dominated the cons and made it a proper system for food production. A major advantage of the hydroponic systems is the plants are subjected to 0 mmol NaCl of growing environment, pH, and nutrient content. It is the best alternative system in areas where in-ground agriculture is difficult. Plants can grow rapidly due to the high availability of O₂ in the root zone. The water and nutrients can be recycled. So, the cost of water and nutrients is lower. The problems related to soil insects and fungi are not present in hydroponics. No problems with the weeds. There is a lot of flexibility in the working conditions. Apart from these pros, there are a few important cons for the hydroponic system. The cost of the system is the biggest problem for the farmer. Both initial and running costs are too high. Lack of knowledge and skill for the operation and maintenance of the system. Though the system is free of soil-borne fungi, bacteria, and insects, several other diseases can spread very rapidly under the hydroponic system (Dunn, 2010). In hydroponics, the infestation of pests and diseases is very low. But the spread of the pathogens is very rapid through the nutrient solution. To reduce the risk of contamination by pathogens, different sterilization techniques like heating and filtering are used in a hydroponic system. Heating the nutrient solution can kill different pathogens in it. The temperature and duration of heating depending on the pathogen species. Filtering can remove pathogens and some other solid particles from the nutrient solution (Son et al., 2019). The nutrient solution is the most important part of the hydroponic system. The solution should contain all essential nutrients for plant growth. Generally, there are 16 essential nutrients which are further divided into macro nutrients, each consisting of >1000 mg/kg dry mass and micronutrients, each consisting of <100 mg/kg dry mass.

The concentrations of different nutrients should be maintained properly. The pH and EC of the nutrient solution are two important things to be monitored and maintained properly (Christie, 2014). Different types of sensors are used in the hydroponic system to maintain important parameters like pH, EC, the temperature at optimum conditions. The water level in the tanks can be measured by ultra-sonic sensors (Son et al., 2019). The nutrient control system which has sensors and controllers is very helpful in operating the hydroponic system (Son et al., 2019).

3. Methods and Materials:

3.1 Plant varieties and cultivation:

In this experiment, two contrasting varieties of sweet potato were grown in the growth chambers of Hans-Ruthenberg-Institute (490) for Tropical Agriculture of the University of Hohenheim from the middle of March to the middle of April 2021. The varieties were CIP 188002.1, tolerant to salinity, and CIP 189151.8, sensitive to salinity.

The seedlings were cut from mother plant vines with a small blade with 2 nodes and a length of 7.5-10 cm. The cuttings were dipped in a biocide solution (Neudorff Spruzit) to prevent damage by bacteria or fungi. The seedlings were planted in a pot (10.5*8.5*13.5 cm) filled with Yoshida nutrient solution. Plants were subjected to 50 mmol NaCl for 14 days from 19 days after planting.

3.2 Experimental setup:

This Experiment was conducted in growth chambers. Pots with a capacity of 1 liter were taken to grow the plants. To support the growing plant, the lid of the pots was given with a plastic staking net. The pots were filled with Yoshida solution. The pH of the solution was monitored every day using a pH meter (pH/cond 3320, Weilheim, Germany). This solution was maintained with optimum pH of 5.8-6.0 using HCL and NaOH solutions. Two growth chambers with 40% and 80% rH were used. As shown in Figure 1, each chamber has 18 pots. Out of 18 pots, 8 pots were of CIP 188002.1, 8 pots were CIP 189151.8, and the remaining 2 pots were blank. In each chamber, four plants from each variety were given 50 mmol of NaCl after 19 days of planting. The other four plants from each variety were considered as plants subjected to 0 mmol NaCl. To provide oxygen to the plant roots, a rubber tube was kept in each pot, and it was provided with air by a motor. This motor was worked at an interval of 15 minutes per every 45 minutes. The experiment was short and ran for 33 days.



Figure 1: Arrangements of the pots in the Growth Chamber.

3.3 Growth chambers:

Two Percival Scientific Plant (PSP) Growth chambers at the Hans Ruthenberg institute at the University of Hohenheim were used for growing the plants. The measurements of the growth chamber are 142 cm wide, 73 cm deep, and 146 cm in length (height). The pots were placed on a rack provided inside at the height of 60 cm from the base. Plant Active Radiation (PAR) was provided by high fluorescent lights present inside the chambers at an intensity of 370-420 µmol m⁻²s⁻¹ (Bottom), 620-670 µmol m⁻²s⁻¹ (Middle), 1010-1040 µmol m⁻²s⁻¹ (Top). The light period was set to 12 hrs, day temperature to 29 °C, and night temperature to 22 °C. Relative air humidity differed in two chambers with 40% and 80%, respectively. Temperature and humidity conditions inside the chambers were monitored with Tiny Tag (Gemini Dataloggers, Chichester, UK) for the experimental period (Figure 2).

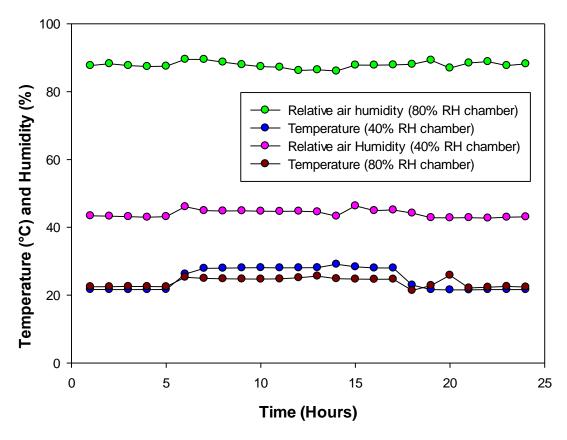


Figure 2: Temperature and Relative Humidity in 40% and 80 % rH climate chambers over one full day.

3.4 Nutrient solution:

The nutrient solution was prepared with the nutrients listed in Table 1. The solution was changed eight times throughout the experiment. The first two times it was changed with seven days intervals. But later it was changed with three days interval. The pH of the solution was measured with a WTW pH 340i instrument. The pH of 5.8-5.9 was maintained. Highly concentrated stock solutions for the entire experiment were prepared for each nutrient in a specific container with deionized water and stored.

Table 1: Compound of stock solutions for the preparation of nutrient solutions (modified after Yoshida et al., 1976).

Label						Final nutrient concentration	
	Element	Chemical	Stock [g/L]	Stock final (ml/L)	Solubility [g/L]	From 1 to 7 DAP	From 8 th day to 32 nd DAP
А							
	N	NH ₄ NO ₃	114.29	1	2089	50%	100%
В	Р	NaH ₂ PO ₄ * 2H ₂ O	50.37	1	850	50%	100%
С	K	K ₂ SO ₄	89.14	1	111	50%	100%
D	Ca	CaCl ₂ * 2H ₂ O	146.73	1	986	50%	100%
E	Mg	MgSO ₄ + 7H ₂ O	405.64	1	710	50%	100%
F	Fe	FeNa – EDTA	15.080	1	N.N	50%	100%
G	Mn	MnCl ₂ * 4H ₂ O	1.875	1	700	50%	100%
	Zn	ZnSO ₄ * 5H ₂ O	0.044		965		
	Cu	CuSO ₄ * 4H ₂ O	0.393		203		
	Мо	(NH ₄) ₆ Mo ₇ O ₂₄ * 4H ₂ O	0.0920		430		
	В	H ₃ BO ₃	11.675		50		

3.5 Transpiration Measuring Chamber:

Total plant transpiration was measured with the aid of a chamber (Figure 3) that allowed assessing transpirational water loss under adjustable climatic conditions (humidity, temperature, and wind speed). Up to 4 plants can be assessed simultaneously with laboratory balances recording weight changes in real-time. Temperature and humidity inside the chamber can be adjusted with heating elements, humidifiers, and dehumidifiers. The voltage of the fans controls the wind speed in the chamber.



Figure 3: Transpiration measuring Chamber used in the experiment.

As shown in Figure 3, the transpiration measurement chamber comprises various parts such as a rotary dryer, a water tank with an ultrasonic humidifier, a mixing chamber, a measuring chamber, and a computer system. The dryer supplies dry air, and the humidifier supplies humidified air into the mixing chamber which contains all heating elements. In the measuring chamber, fans provide for homogenous air conditions and balances monitor the plant weight and store the values on the computer. Sensors between the mixing and the measuring chamber send values in user-defined intervals (here every second) to the central system which controls the heating elements and humidifiers. If the desired humidity is very high, additional moist air from the exhaust outlet of the rotary drier is used to reach the desired humidity.

In the experiment reported here, four plants were measured simultaneously (Figure 4) of which two were subjected to salinity and two served as non-stressed plants. The top of the pots was covered with aluminium foil to avoid evaporation. The temperature was set around 29-30° C. The plants were tested under four levels of relative humidity 30%, 45%, 60%, and 75%, respectively. The rH levels were later transformed to VPD resulting in 3.33 kPa, 2.77 kPa, 2.18 kPa, and 1.66 kPa, respectively. The order of the humidity level was based on the relative humidity of the growth chamber plants were grown in. Plants grown under 80% rH were tested in the order of 30%, 45%, 60%, and 75%,

respectively, and plants grown under 40% rH were tested in the order of 75%, 60%, 45%, and 30%, respectively. Plants were also tested with a sudden change in the rH directly from 75% to 30% and vice versa.

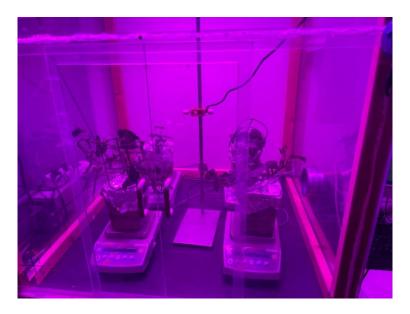


Figure 4: Plants in the measuring chamber during the measurement process.

3.6 Measurements:

3.6.1 Daily water loss:

Water loss from the pots was measured for 32 days, starting from the first day of the experiment to the last day. The recording was done every day at 11 am. A balance (KERN & Sohn GmbH, Balingen) was used to weigh the pots. Daily water loss was determined as the daily difference in weight between the pot weight before watering and the pot target weight. From this daily water loss data, the cumulative water loss was calculated by adding up everyday water loss from the second day to the last day of the experiment.

The transpiration rate was also calculated from this daily water loss data and leaf area. This rate defines the milli moles of water lost per square meter leaf area per second (mmol.m⁻².s⁻¹). This was calculated using the following formula.

TR= (WL/18)*1000/((LA/10000)*T)

TR: Transpiration Rate (m mol/m²/s)

WL: Water loss (g)

LA: Leaf area (cm²)

T: Time (seconds)

3.6.2 Leaf Area:

The leaf area of the plants was measured from the 18th day after planting to the 32nd day, the last day

of the experiment with the smartphone app "easy leaf area". During the experimental period, the area

of 11 leaves, from the first leaf at the basal part of the vine to the 11th, the most apical fully developed

leaf was recorded for each plant. Leaf area for emerging leaves was measured until the area was

constant for two consecutive days which defined the leaf as fully expanded. The final leaf area was

measured on the last day of the experiment with a leaf surface measuring device (LI-COR Inc., LI 3000C,

USA).

3.6.3 Transpiration:

The transpiration of the plants was measured in the Transpiration measuring chamber (TMC). The

plants in each growth chamber were measured on every alternate day. The measurements were

started 20 days after planting and from the 2nd day of salt application with the plants in the 80% rH

condition. This process was continued for 14 days. Total of seven days and seven observations for

each chamber. Out of these seven observations, five proper observations were considered for the

data analysis. On each day, four cycles were run in the TMC. Each cycle consists of four plants, two

were plants subjected to 0 mmol NaCl and two were 50 mmol NaCl. The amount of water transpired

by the plants at the four levels of relative humidity (30%, 45%, 60%, 75%) was recorded by the

computer. These levels after converting them into VPD (kPa) were 3.31 ± 0.05 , 2.77 ± 0.02 , 2.18 ± 0.04

and 1.7 ± 0.06 respectively. The plants grown in the dry conditions were exposed to wet conditions in

the measuring chamber initially and plants grown in the dry chamber were exposed to wet conditions.

The water loss was recorded in grams, and it was converted as transpiration rate (m mol/m²/s) by

using the above-mentioned formula. The relative humidity levels were calculated into Vapour

pressure deficit levels (VPD) by using the following formula.

VPD = Es(T) [kPa] - Ea

VPD: Vapour pressure deficit

Es(T): Saturation vapour pressure [kPa]

Ea: Actual vapour pressure

 $es(T) = 611exp \frac{17.27T}{237.3+T}$ (Wilhelm, 1975)

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Es(T): Saturation vapour pressure [kPa]

611exp: exponential constant

T: Temperature

$$Ea = RH \frac{Es(T)}{100}$$

Ea: Actual vapor pressure

RH: Relative Humidity

Es(T): Saturation vapour pressure [kPa]

After general transpiration measurement at four VPD levels, VPD had suddenly changed to a low level and then to a high level again for plants grown in 40% rH conditions. VPD had suddenly changed to a high level and then to a low level again for plants grown in 80% rH conditions. The responses of plants at both high and low VPD levels were recorded and shown in Figure 9. The VPD levels were calculated into kPa. They were 3.37 ± 0.08 at a high level and 1.82 ± 0.07 at a low level.

3.6.4 Biomass:

The fresh weight and dry weight of the leaf, petiole, shoot, and root were measured using the balance. The leaves biomass of the 11 leaves was collected individually and measured. The shooting part was divided into three parts, bottom, middle, and top. Then they were measured separately. The biomass of the remaining leaves and roots was also collected and measured separately. The plants were dried in the hot air oven for one week after the final harvesting.

3.6.5 Vine length:

The Vine length was measured using a ruler immediately after harvesting the plants.

3.7 Statistical Analysis:

For statistical analysis, the raw data was sorted in Microsoft Excel. The means, standard deviation, and standard error were calculated in Microsoft Excel. The graphs were made using the Sigma plot 12.5 software. There were four replications. So, the values were calculated on the average of the replications. Standard error was calculated using the formula:

SE= SD/Sqrt of number of observations

SE = Standard error, SD: Standard Deviation Sqrt: Square root

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Both positive and negative error bars were plotted in the graphs. The standard error was used to plot the standard error. A significant difference in means of leaf area, transpiration rates between the treatments was tested via student t-test at $p \le 0.05$.

4. Results:

4.1 Leaf area:

All the plants in both 40% RH and 80% RH chambers had stopped leaf development after 25 days of planting, 6 days of salt treatment. Still, measurements were continued for one more week. There was no considerable change in that one week. This is clearly shown in Figure 5.

Leaf area throughout the experiment was neither significantly different between the varieties nor air humidity conditions. In 40% rH conditions, for the variety CIP188002.1, plants subjected to 0 and 50 mmol NaCl had started with the leaf area of 217 ± 15 cm² and 182 ± 22 cm² and ended with 363 ± 16 cm² and 324 ± 17 cm², respectively. For the variety CIP189151.8, the leaf area of the plants subjected to 0 and 50 mmol NaCl in the beginning and ending was 147 ± 26 cm² and 159 ± 11 cm² and 233 ± 9 cm² and 241 ± 19 cm². The variety CIP188002.1 has better leaf development compared to the CIP189151.8. Though salinity did not affect leaf area, CIP188002.1 had reported a higher leaf area than CIP189151.8. The final leaf area of variety CIP188002.1 was reached to 363 ± 16 cm² in plants subjected to 0 mmol NaCl and 324 ± 17 cm² in 50 mmol NaCl. But for CIP189151.8, the leaf area was ended with 233 ± 9 cm² and 241 ± 19 cm² in plants subjected to 0 and 50 mmol NaCl, respectively.

In 80% rH conditions, for the variety CIP188002.1, plants subjected to 0 and 50 mmol NaCl had similar leaf area development for the first five days after the salt treatment. Later, the plants subjected to 50 mmol NaCl had higher leaf area development than the plants subjected to 0 mmol NaCl. Though the plants subjected to 0 mmol NaCl and 50 mmol NaCl started with leaf area of 262 ± 14 cm² and 257 ± 33 cm² respectively, they ended with the leaf area of 437 ± 15 cm² and 413 ± 27 cm² respectively. For the variety CIP189151.8, plants subjected to 0 and 50 mmol NaCl showed similar leaf area development for the first two days after the salt treatment. But from the 3^{rd} day of salt treatment, the leaf area of plants subjected to 0 mmol NaCl was raised significantly than the plants subjected to 50 mmol NaCl. Leaf area of the plants subjected to 0 and 50 mmol NaCl on the first day of salt treatment was 124 ± 18 cm² and 133 ± 30 cm², but on the last day of the experiment was 284 ± 26 cm² and 213 ± 24 cm², respectively. Same as plants grown in 40% rH, the variety CIP188002.1 had a higher leaf area than CIP 189151.8 in both treatments. For CIP188002.1, leaf area in plants subjected to 0 and 50 mmol NaCl was reached to 413 ± 27 cm² and 437 ± 15 cm², respectively. For CIP189151.8, the final leaf area of plants subjected to 0 and 50 mmol NaCl was 284 ± 26 cm², respectively.

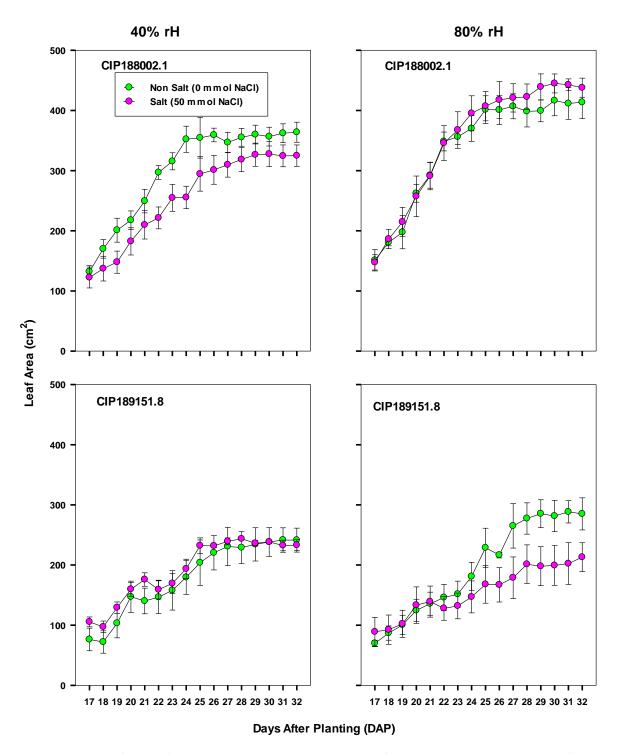


Figure 5: Leaf area of plants subjected to 0 and 50 mmol for two sweet potato varieties (CIP 188002.1 and CIP189151.8) grown in climate chambers set to 40% and 80% rH respectively.

4.2 Transpiration Rate:

In 40% rH conditions, salinity had no significant effect on the transpiration rate for the variety CIP188002.1 (p=0.4919, p>0.05). The transpiration rate in plants subjected to 0 and 50 mmol NaCl on the day before the salt application was 1.68 ± 0.36 and 1.84 ± 0.27 mmol.m⁻².s⁻¹ respectively. But, the day after the salt application, the transpiration rate was dropped to 1.15 ± 0.1 mmol.m⁻².s⁻¹ in plants subjected to 50 mmol NaCl. Not only in plants subjected to 50 mmol NaCl but also in the plants subjected to 0 mmol NaCl, the transpiration rate was also decreased to 1.04 ± 0.05 mmol.m⁻².s⁻¹. Then there was a slight increase in both 0 and 50 mmol, but it gradually decreased again. There was a sudden increase on, the 32nd day, the last day after planting in both the plants. it was ended with 1.27 ± 0.16 and 1.21 ± 0.16 mmol.m⁻².s⁻¹ in plants subjected to 0 and 50 mmol NaCl, respectively. Salinity had a significant effect on the transpiration rate for the variety CIP189151.8 (p=0.0106, p<0.05). Plants subjected to 0 mmol NaCl $(1.5 - 2.0 \text{ mmol.m}^{-2}.\text{s}^{-1})$ had higher transpiration rate than 50 mmol NaCl $(1.0 - 1.5 \text{ mmol.m}^{-2}.\text{s}^{-1})$. Though the plants subjected to 50 mmol NaCl increased the rate from 1.31 ± 0.1 to 1.74 ± 0.52 mmol.m⁻².s⁻¹, but later it was decreased to 1.0 ± 0.08 mmol.m⁻².s⁻¹. Then, it was increased slightly and ended with 1.24 ± 0.311 mmol.m⁻².s⁻¹. Plants subjected to 0 mmol NaCl had the highest transpiration rate on the 24th and 26th days after planting but later it was decreased and ended with 1.61 ± 0.24 mmol.m⁻².s⁻¹. Salinity had shown a significant effect on transpiration rate for the variety CIP189151.8 but not for CIP188002.1. For variety CIP188002.1, the transpiration rate of plants subjected to 0 and 50 mmol NaCl on the last day (32nd Day) of the experiment was 1.27 ± 0.16 and 1.21 ± 0.16 mmol.m⁻².s⁻¹, respectively. For variety CIP189151.8, the transpiration rate of the plants subjected to 0 and 50 mmol NaCl on the last day (32^{nd} Day) of the experiment was 1.61 \pm 0.24 and 1.24 ± 0.31 mmol.m⁻².s⁻¹, respectively.

In 80% rH conditions, plants subjected to 0 and 50 mmol NaCl of the variety CIP188002.1 (p=0.8992, p>0.05) and CIP 189151.8 (p=0.9188, p>0.05) reported similar transpiration rate (Figure 6). Salinity had no significant effect on the transpiration rate in both varieties. For CIP188002.1, after the salt application, the transpiration rate was dropped from 1.04 ± 0.21 to 0.25 ± 0.03 mmol.m⁻².s⁻¹. Then, it was maintained at the same level till the end and recorded 0.24 ± 0.05 mmol.m⁻².s⁻¹ on the last day. Under non-stressed condition for CIP188002.1 (0.59 ± 0.22 mmol.m⁻².s⁻¹) and CIP189151.8 (1.69 ± 0.44 mmol.m⁻².s⁻¹), maximum transpiration rate was observed at 20 DAP. transpiration rate was declined from 1.69 ± 0.44 to 0.54 ± 0.04 mmol.m⁻².s⁻¹ from 20th to 24th day after planting. Then, it was maintained at the same level and on the last day (32^{nd} day) of the experiment, it was 0.66 ± 0.13 . In plants subjected to 50 mmol, NaCl transpiration rate had decreased from the next day of salt application. It was declined from 1.20 ± 0.33 to 0.72 ± 0.22 mmol.m⁻².s⁻¹, then it was maintained the

same level from 24^{th} to 28^{th} day after planting. Then, there was a decline in the rate and on the last day (32^{nd} day), it was 0.52 ± 0.11 mmol.m⁻².s⁻¹. Both the plants subjected to 0 and 50 mmol NaCl of the variety CIP188002.1 reported a lesser transpiration rate than the variety CIP189151.8. The transpiration rate of the plants subjected to 0 and 50 mmol NaCl of the variety CIP188002.1 on the last day of the experiment was 0.30 ± 0.11 and 0.24 ± 0.05 mmol.m⁻².s⁻¹, respectively. For the variety CIP189151.8, the transpiration rate of the plants subjected to 0 and 50 mmol NaCl was 0.66 ± 0.13 and 0.52 ± 0.11 mmol.m⁻².s⁻¹, respectively.

The transpiration rate was very low in the plants grown in the 80% rH conditions compared to the plants grown in 40% rH conditions. The difference was high in the last 10 days of the experiment. In 40% rH conditions, the transpiration rate of the plants subjected to 0 and 50 mmol NaCl on the last day of the experiment was 1.2 to 1.6 and 1.2 to 1.25 mmol.m⁻².s⁻¹, respectively. But in the case of 80% rH conditions, the transpiration rate of the plants subjected to 0 and 50 mmol NaCl on the last day of the experiment was 0.3 to 0.6 and 0.2 to 0.5 mmol.m⁻².s⁻¹, respectively.

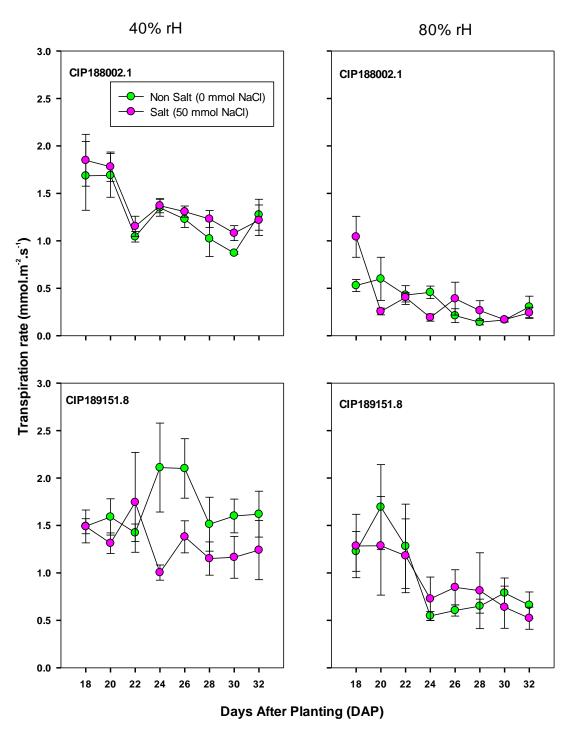


Figure 6: Transpiration Rate of plants subjected to 0 and 50 mmol NaCl of both varieties CIP 188002.1 and CIP189151.8 under 40% and 80% rH conditions.

4.3 Cumulative Water loss:

Cumulative water loss throughout the experiment was similar for all plants grown in 40% rH conditions and did neither significantly differ between varieties nor treatments (Figure 7). Salinity had no effect in both varieties and under both humidity conditions. The humidity had a significant effect on the cumulative water loss. There was a significant difference in cumulative water loss between the two humidity conditions. Plants grown in 40% rH condition had higher water loss (1300-1500 g) than the plants grown in 80% rH condition (700-900 g).

In 40% rH conditions, for the variety CIP188002.1, cumulative water loss in the plants subjected to 0 and 50 mmol NaCl on the next day of salt treatment (20th day) was 323.19 g and 343.69 g, respectively. On the last day of the experiment, it was 1490.83 g in plants subjected to 0 mmol NaCl and 1329.99 g in plants subjected to 50 mmol NaCl. In the variety CIP189151.8 also, plants subjected to 0 and 50 mmol NaCl reported a similar amount of water loss from the day of the salt treatment to the end of the experiment. Cumulative water loss of the plants subjected to 0 and 50 mmol NaCl on the 20th day after planting was 325.17 g and 325.17 g of cumulative water loss, respectively. On the 26th day of planting, the plants subjected to 0 and 50 mmol NaCl reported 917.34 g and 892.52 g, respectively. Then on the last day of the experiment, the plants subjected to 0 and 50 mmol NaCl were ended with 1472.65 g and 1387.62 g respectively. In the case of comparison of both varieties, CIP188002.1 had a higher cumulative water loss in plants subjected to 0 mmol NaCl (1490.83 g). But the variety CIP 189151.8 had a higher cumulative water loss in plants subjected to 50 mmol NaCl (1387.62 g).

In 80% rH conditions, for the variety CIP188002.1, cumulative water loss of the plants subjected to 0 and 50 mmol NaCl on the 20th day after planting was 188.27 g and 206.51 g, respectively. Though there was a similar amount of water loss on the 22nd and 24th DAP in both the treatments. Later, plants subjected to 50 mmol NaCl had lost more water than 0 mmol NaCl and recorded 762.67 g on the last day of the experiment. Plants subjected to 0 mmol NaCl on the last day of the experiment reported 724.68 g of water loss. Both 0 and 50 mmol NaCl subjected plants of the variety CIP189151.8 showed similar cumulative water loss from the next day of the salt application. But, in the end, the plants subjected to 0 mmol NaCl had higher water loss than the plants subjected to 50 mmol NaCl. On the day after the salt application (20 DAP), cumulative water loss in plants subjected to 0 and 50 mmol NaCl was 206.51 g and 275.8 g respectively. On the 26th day of planning, both the treatments reported a similar amount of water loss, 467.79 g and 466.22 g in plants subjected to 0 and 50 mmol NaCl, respectively. Later, water loss was higher for plants subjected to 0 mmol NaCl than 50 mmol NaCl. On the last day of the experiment, plants subjected to 0 and 50 mmol NaCl reported 861.53 g and 760.46 g, respectively.

In the comparison of both the varieties, plants subjected to 50 mmol NaCl reported a similar amount of water loss in both the varieties, 762.67 g and 760.46 g for CIP188002.1 and CIP189151.8, respectively. In the case of 0 mmol NaCl, the variety CIP189151.8 had a higher cumulative water loss (861.53g) than the variety CIP188002.1 (724.68g).

In 40% rH conditions, plants subjected to 0 mmol NaCl had a higher water loss in both the varieties (1490.83 for CIP188002.1 and 1472.65 for CIP189151.8). But, in 80% rH conditions, plants subjected to 50 mmol NaCl had a higher water loss in CIP 188002.1 (762.67 g) and plants subjected to 0 mmol NaCl had a higher water loss in the variety CIP189151.8 (861.53 g).

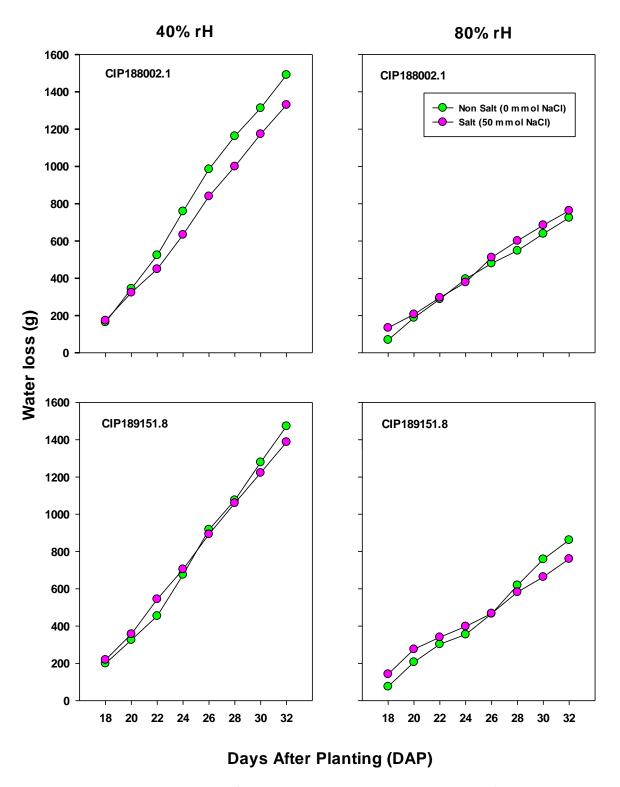


Figure 7: Cumulative Water loss of plants subjected to 0 and 50 mmol NaCl of both varieties CIP 188002.1 and CIP189151.8 in 40% and 80% rH conditions.

4.4 VPD vs Transpiration:

4.4.1 40% rH condition:

Plants subjected to 0 mmol NaCl of the variety CIP188002.1 reported the highest transpiration on day 1 among five days (Figure 8). Transpiration on day 1 at four levels of the VPD was 2.36 ± 0.11, 5.21 ± 0.19, 7.64 ± 0.10 and 8.85 ± 0.22 mmol.m⁻².s⁻¹at 1.7, 2.18, 2.77 and 3.31 kPa respectively. Day 1 was followed by Day 7, day 3, day 5, and day 9, respectively. The transpiration on the last day was very low compared to all other days. The transpiration on the last day of the measurements was 1.70 ± 0.15 , 4.25 ± 0.08 , 5.39 ± 0.08 and 6.91 ± 0.11 mmol.m⁻².s⁻¹ respectively. In the plants subjected to 50 mmol NaCl of the variety CIP188002.1, transpiration was highest on day 7 with 3.84 ± 0.11 , 6.47 ± 0.14 , 8.92 ± 0.12 and 9.80 ± 0.12 mmol.m⁻².s⁻¹at 1.7, 2.18, 2.77 and 3.31 kPa respectively. Day 7 was followed by day 3, day 1, day 5, and day 9, respectively. The transpiration on day 9 was lower than all other days. The values on day 9 were 1.21 \pm 0.10, 3.65 \pm 0.15, 5.91 \pm 0.09 and 8.25 \pm 0.11, respectively. In an overall comparison of the plants subjected to 0 and 50 mmol, NaCl of the variety CIP188002.1, the plants subjected to 50 mmol NaCl had higher transpiration at the four levels of VPD than the plants subjected to 0 mmol NaCl. The transpiration at VPD 3.31 kPa on day 1 in plants subjected to 0 and 50 mmol NaCl was 8.85 ± 0.22 and 9.80 ± 0.12 mmol.m⁻².s⁻¹, respectively. On day 9, at the same VPD level, these values were 6.91 ± 0.11 and 8.25 ± 0.11 mmol.m⁻².s⁻¹ in plants subjected to 0 and 50 mmol NaCl, respectively. Though the lower transpiration days were day 5 and day 9 in both the treatments, the highest transpiration was on day 1 in the plants subjected to 0 mmol NaCl and day 7 in plants subjected to 50 mmol NaCl.

In the case of the variety CIP189151.8, the plants subjected to 0 mmol NaCl showed the highest transpiration on day 1 followed by day 3, day 9, day 5, and day 7. The transpiration on the day 1 was 4.33 ± 0.14 , 7.75 ± 0.16 , 10.49 ± 0.14 and 12.55 ± 0.13 mmol.m⁻².s⁻¹at 1.7, 2.18, 2.77 and 3.31 kPa, respectively. There was a considerable difference between day 1 and day 3. The transpiration on day 3 was 3.97 ± 0.14 , 6.37 ± 0.24 , 8.83 ± 0.19 and 9.90 ± 0.35 mmol.m⁻².s⁻¹at 1.7, 2.18, 2.77 and 3.31 kPa, respectively. Lowest transpiration was observed on Day 7 with 2.98 ± 0.25 , 5.36 ± 0.08 , 7.01 ± 0.08 , 8.19 ± 0.22 mmol.m⁻².s⁻¹ respectively. In the plants subjected to 50 mmol NaCl of the variety CIP189151.8, transpiration was highest on day 1 followed by day 3, day 5, day 7, and day 9, respectively. Transpiration on day 1 was 2.48 ± 0.18 , 6.8 ± 0.24 , 10.37 ± 0.15 and 12.25 ± 0.11 mmol.m⁻².s⁻¹at 1.7, 2.18, 2.77 and 3.31 kPa respectively. The transpiration on the last day was lower than all other days. it was 1.62 ± 0.09 , 3.95 ± 0.20 , 5.29 ± 0.12 and 6.82 ± 0.16 mmol.m⁻².s⁻¹at 1.7, 2.18, 2.77 and 3.31 kPa, respectively. The plants subjected to 0 and 50 mmol NaCl of variety CIP189151.8 have shown similar transpiration compared to each other. The highest transpiration in plants subjected to

0 and 50 mmol NaCl was observed on day 1, but the lower transpiration was observed on day 7 and day 9, respectively.

The variety CIP189151.8 had higher transpiration than CIP188002.1 and CIP189151.8. In CIP189151.8, transpiration at VPD level, 3.31 kPa, was reached to 12.55 ± 0.13 and 12.25 ± 0.11 mmol.m⁻².s⁻¹ in plants subjected to 0 and 50 mmol NaCl, respectively. But in CIP188002.1, it was only 8.85 ± 0.22 and 10.04 ± 0.11 mmol.m⁻².s⁻¹ in plants subjected to 0 and 50 mmol NaCl, respectively. The lowest transpiration was observed on day 9 in plants subjected to 50 mmol NaCl of both varieties. But, in plants subjected to 0 mmol NaCl, it was day 9 and day 7 for the varieties CIP18002.1 and CIP189151.8 respectively.

4.4.2 80% RH Chamber:

Transpiration was measured on day 2, day 4, day 6, day 8, and day 10. Plants subjected to 0 mmol NaCl of the variety CIP188002.1 showed the highest transpiration on day 2 followed by day 10, day 6, day 8, and day 4. The transpiration on day 2 was 6.44 ± 0.39 , 4.81 ± 0.06 , 2.99 ± 0.06 and 2.89 ± 0.09 mmol.m $^{-2}$.s $^{-1}$ on VPD levels of 3.31 ± 0.05, 2.77 ± 0.02, 2.18 ± 0.04 and 1.7 ± 0.06 kPa, respectively. The lower transpiration was observed on day 4 compared to other days. it was 4.90 ± 0.10, 2.87 ± 0.04, 2.51 ± 0.03 and 2.57 ± 0.09 mmol.m⁻².s⁻¹, respectively. Though day 10 had high transpiration after day 2, it was in the last position with lower transpiration, 4.75 ± 0.08 mmol.m⁻².s⁻¹ at high VPD level, 3.31 kPa. In the plants subjected to 50 mmol NaCl of the variety CIP188002.1, transpiration was highest on day 2 followed by day 6, day 10, day 8, and day 4. Transpiration on day 2 was 7.15 ± 0.16 , 4.77 ± 0.10 , 2.62 ± 0.10 and 2.55 ± 0.12 mmol.m⁻².s⁻¹ at 3.31 ± 0.05 , 2.77 ± 0.02 , 2.18 ± 0.04 and 1.7 ± 0.06 kPa, respectively. Lower transpiration was observed on day 4. It was 4.32 ± 0.04 , 2.82 ± 0.06 , 1.81 ± 0.04 and 1.70 ± 0.07 mmol.m⁻².s⁻¹ at respective VPD levels. Plants on day 10 showed higher transpiration at 2.18 ± 0.04 and 1.7 ± 0.06 kPa but showed lower at 3.31 ± 0.05 , 2.77 ± 0.02 kPa VPD levels. The transpiration was similar in plants subjected to 0 and 50 mmol NaCl of the variety CIP188002.1. Though both the treatments had higher transpiration on day 2, the lower transpiration was on day 10 and day in plants subjected to 0 and 50 mmol NaCl, respectively (Figure 8).

In the case of the variety CIP189151.8, plants subjected to 0 mmol NaCl showed the highest transpiration on day 2 followed by day 4, day 8, day 10, and day 6, respectively. Transpiration on day 2 was 8.6738 ± 0.2204 , 6.32 ± 0.13 , 5.06 ± 0.14 and 5.87 ± 0.28 mmol.m⁻².s⁻¹on VPD levels of 3.31 ± 0.05 , 2.77 ± 0.02 , 2.18 ± 0.04 and 1.7 ± 0.06 kPa, respectively. Lower transpiration was observed on day 6 compared to all other days. It was 4.55 ± 0.05 , 5.22 ± 0.10 , 4.7 ± 0.04 and 4.32 ± 0.29 mmol.m⁻².s⁻¹ on the respected VPD levels. Transpiration on day 8 was high at VPD 3.31 ± 0.05 and 2.77 ± 0.02 kPa but it was lower at other VPD levels 2.18 ± 0.04 and 1.7 ± 0.06 kPa. In plants subjected to 50 mmol

NaCl of variety CIP189151.8, the higher transpiration was observed on day 2 followed by day, day 8, day 10, and day 6. Transpiration on day 2 was 9.81 ± 0.24 , 5.31 ± 0.16 , 2.91 ± 0.20 and 3.57 ± 0.26 mmol.m⁻².s⁻¹ at 3.31 ± 0.05 , 2.77 ± 0.02 , 2.18 ± 0.04 and 1.7 ± 0.06 kPa, respectively. The lower transpiration was recorded on day 4. It was 8.37 ± 0.10 , 5.29 ± 0.16 , 2.83 ± 0.19 and 2.27 ± 0.26 mmol.m⁻².s⁻¹ at respected VPD levels. The plants subjected to 0 and 50 mmol NaCl reported the same order for the hierarchy of transpiration, day 2, day 4, day 8, day 10, and day 6. The plants subjected to 0 mmol NaCl showed higher transpiration than plants subjected to 50 mmol NaCl at 1.7 ± 0.06 kPa VPD level. But the plants subjected to 50 mmol NaCl had higher transpiration than plants subjected to 0 mmol NaCl at 3.31 ± 0.05 kPa VPD level.

The variety CIP189151.8 had higher transpiration than the CIP188002.1. Transpiration of CIP188002.1 at 3.31 ± 0.05 kPa VPD level was 6.44 ± 0.39 mmol.m⁻².s⁻¹ and 7.15 ± 0.16 mmol.m⁻².s⁻¹ in plants subjected to 0 and 50 mmol NaCl. In the variety CIP189151.8 at the same level of VPD, the transpiration was 8.67 ± 0.22 mmol.m⁻².s⁻¹ and 9.81 ± 0.24 mmol.m⁻².s⁻¹ in plants subjected to 0 and 50 mmol NaCl. Both treatments of two varieties had higher transpiration on day 2 and lower transpiration was on day 6 in both plants subjected to 0 and 50 mmol NaCl for the variety CIP188002.1 and day 10 in plants subjected to 0 mmol NaCl and day 4 in plants subjected to 50 mmol NaCl for the variety CIP189151.8.

Plants grown in the 40% rH conditions had higher transpiration than the plants grown in the 80% rH conditions. The highest transpiration was nearly 13 mmol.m⁻².s⁻¹ and 10 mmol.m⁻².s⁻¹ in the plants grown in 40% and 80% rH conditions, respectively. The highest transpiration was observed on the first day of the measurements in both chambers. The lowest transpiration day differed between the two chambers.

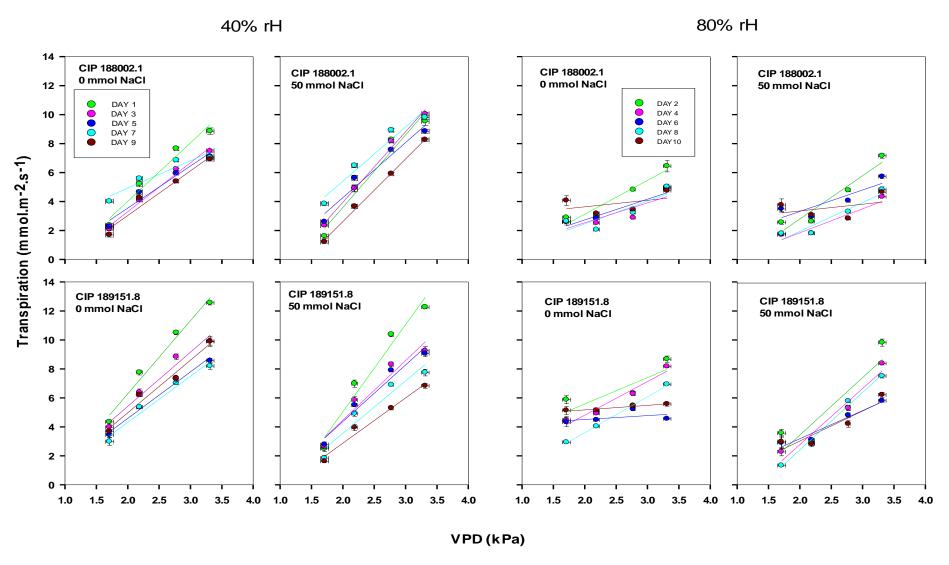


Figure 8: Transpiration of plants subjected to 0 and 50 mmol NaCl of both varieties CIP 188002.1 and CIP189151.8 at four different VPD levels.

(Measurements for 40% rH and 80 % rH conditions were started with low VPD and high VPD, respectively)

4.5 Dry weight:

Dry matter accumulation throughout the experiment was similar for all plants grown under non-saline conditions (4-4.8 g per plant) and did neither significantly differ between varieties nor air humidity conditions. For susceptible CIP 189151.8, salinity significantly reduced dry matter accumulation for both humidity conditions with the effect being stronger in 80% rH conditions than 40% rH conditions, whereas in tolerant CIP 188002.1 salinity did not affect dry matter accumulation (Table 2). For CIP 189151.8, dry weight of the plants subjected to 50mmol NaCl in 40% and 80% rH conditions was 3.66 ± 0.29 and 2.47 ± 0.39 , respectively. For CIP 188002.1, it was 4.45 ± 0.27 and 4.56 ± 0.46 in 40% and 80% rH conditions, respectively.

Table 2: Dry weight of plants subjected to 0 and 50 mmol NaCl of both varieties CIP 188002.1 and CIP189151.8 in 40% and 80% rH conditions.

Conditions	40 % rH				80% rH			
Variety	CIP188002.1		CIP189151.8		CIP188002.1		CIP189151.8	
Character	Dry	SE	Dry	SE	Dry	SE	Dry	SE
	weight		weight		weight		weight	
Treatment								
Salt (50	4.45	0.27	3.66	0.29	4.56	0.46	2.47	0.39
mmol NaCl)								
Non-Salt (0	4.68	0.11	4.42	0.41	5.17	0.45	3.89	0.62
mmol NaCl)								
Significance	NS		NS		NS		*	

Note: p < 0.05 NS: Non-Significant *Significant SE: Standard Error rH: Relative Humidity

For tolerant CIP 188002.1, salinity did not affect the length of the vine in both humidity conditions. In the case of sensitive CIP189151.8, salinity had a significant effect in 80% rh conditions but not in 40% rH conditions. Though CIP189151.8 was sensitive, it had a higher vine length than the tolerant variety, CIP188002.1. The highest vine length (43.62 ± 7.30) was recorded for plants subjected to 0 mmol NaCl in 40% rH conditions. The lowest (7.27 ± 1.44) was for plants subjected to 50 mmol NaCl in 80% rH conditions (Table 3).

Table 3: Vine length of plants subjected to 0 and 50 mmol NaCl of both varieties CIP 188002.1 and CIP189151.8 in 40% and 80% rH conditions.

Conditions	40 % rH				80% rH			
Variety	CIP188002.1		CIP189151.8		CIP188002.1		CIP189151.8	
Character	Length	SE	Length	SE	Length	SE	Length	SE
	of the		of the		of the		of the	
	vine		vine		vine		vine	
Treatment								
Salt (50	27	4.94	30.82	4.37	23.82	3.98	7.27	1.44
mmol NaCl)								
Non-Salt (0	22.55	1.53	43.62	7.30	18	2.12	35.37	10.12
mmol NaCl)								
Significance	NS		NS		NS		*	

Note: Note: p ≤ 0.05 NS: Non-Significant *Significant SE: Standard Error rH: Relative Humidity

4.6 Sudden change of VPD from low to high and high to low:

In 40% rH conditions, plants subjected to 0 mmol NaCl of the variety CIP188002.1 showed the highest transpiration on Day 7 followed by day 1, day 5, day 3, and day 9 at low VPD level, 1.82 ± 0.07 kPa (Figure 9). The transpiration on day 7 was 3.96 ± 0.12 and on day 9 was 2.33 ± 0.19 mmol.m⁻².s⁻¹. The order was changed at a high VPD level. The highest transpiration at a high VPD level was observed on day 1 followed by day 9, day 7, day 5, and day 3, respectively. Transpiration on day 1 was 8.97 ± 0.72 and on day 3 was 7.39 ± 0.35 mmol.m⁻².s⁻¹ at 3.37 ± 0.08 kPa VPD. Plants subjected to 50 mmol NaCl of the variety CIP188002.1 at low VPD level reported the highest transpiration on day 9 followed by day 5, day 3, day 7, and day 1, respectively. Transpiration on day 9 was 5.86 ± 0.41 mmol.m⁻².s⁻¹ and on day 1 was 3.51 ± 0.36 mmol.m⁻².s⁻¹. Transpiration was highest on day 9 followed by day 3, day 1, day 7, and day 5, respectively at a high VPD level of 3.37 ± 0.08 kPa. Transpiration on day 9 was 11.07 ± 0.46 mmol.m⁻².s⁻¹ and on day 5 was 4.51 ± 0.07 mmol.m⁻².s⁻¹. The highest transpiration at both high and low VPD levels was observed on Day 9. Plants subjected to 50 mmol NaCl at both high and low VPD levels. The highest transpiration in plants subjected to 50 and 0 mmol NaCl at low and high VPD level was 5.86 ± 0.41 and 11.07 ± 0.46 mmol.m⁻².s⁻¹ and 3.85 ± 0.35 and 8.97 ± 0.72 mmol.m⁻².s⁻¹, respectively.

In the variety CIP189151.8, the plants subjected to 0 mmol NaCl showed the highest transpiration on day 1 followed by day 3, day 7, day 5, and day 9, respectively at both low and high VPD levels. The transpiration on day 1 at low and high VPD levels was 6.60 ± 0.30 and 12.47 ± 0.64 mmol.m⁻².s⁻¹, respectively. There was a considerable difference between day 5 and day 9 compared to other days. Transpiration on day 5 and day 9 at low and high VPD levels was 4.35 ± 0.29 and 8.52 ± 0.26 mmol.m⁻².s⁻¹ and 2.61 ± 0.12 and 7.51 ± 0.23 mmol.m⁻².s⁻¹, respectively. Plants subjected to 50 mmol NaCl of the variety CIP189151.8 reported the highest transpiration on day 1 followed by day 3, day 5, day 7, and day 9 at low VPD. Day 7 had the lowest transpiration instead of day 9 at a high VPD level. Transpiration at low and high VPD levels on day 1 was 4.86 ± 0.35 and 10.03 ± 0.54 mmol.m⁻².s⁻¹, respectively.

Though the transpiration on day 1 at high VPD level was 10.03 ± 0.54 mmol.m⁻².s⁻¹ but the transpiration on day 3 was only 7.89 ± 0.49 mmol.m⁻².s⁻¹ which was the second-highest transpired day. The other days were almost like day 3 at a high VPD level. Transpiration on day 9 at low VPD level was 2.93 ± 0.27 mmol.m⁻².s⁻¹ and on day 7 at high VPD level was 6.85 ± 0.40 mmol.m⁻².s⁻¹. In between plants subjected to 0 and 50 mmol NaCl of the variety CIP189151.8, 0 mmol NaCl had higher transpiration than the 50 mmol NaCl both at low and high VPD levels. The highest transpiration in plants subjected to 0 and 50 mmol NaCl at low and high VPD level was 6.60 ± 0.30 and 12.47 ± 0.64 mmol.m⁻².s⁻¹ and 4.86 ± 0.35 and 10.03 ± 0.54 mmol.m⁻².s⁻¹, respectively.

Plants subjected to 0 mmol NaCl of variety CIP189151.8 had higher transpiration at low and high VPD levels than variety CIP188002.1. The highest transpiration in plants subjected to 0 mmol NaCl of varieties CIP189151.8 and CIP1880022.1 at low and high VPD levels was 6.60 ± 0.30 and 12.47 ± 0.64 mmol.m⁻².s⁻¹ and 3.96 ± 0.12 and 8.97 ± 0.72 mmol.m⁻².s⁻¹, respectively. But in the case of plants subjected to 50 mmol NaCl, the variety CIP188002.1 had shown higher transpiration than the CIP189151.8. The highest transpiration in plants subjected to 50 mmol of NaCl of varieties CIP188002.1 and CIP189151.8 at low and high VPD levels was 5.86 ± 0.41 and 11.07 ± 0.46 mmol.m⁻².s⁻¹ and 4.86 ± 0.35 54 and 10.03 ± 0.54 mmol.m⁻².s⁻¹, respectively.

In the 80% rH chamber, plants were subjected to high VPD (3.37 \pm 0.08 kPa) at first then sudden change to low VPD (1.82 \pm 0.07 kPa). Plants subjected to 0 mmol NaCl of the variety CIP188002.1 had shown the highest transpiration on day 2 followed by day 6, day 8, day 10, and day 4 at high VPD level and day 2, day 6 day 10, day 8, and day 4 at low VPD level, respectively. Transpiration at high and low VPD levels on day 2 was 7.41 \pm 0.29 and 3.28 \pm 0.31 mmol.m⁻².s⁻¹, respectively. Transpiration on day 4 was 5.77 \pm 0.17 mmol.m⁻².s⁻¹ at high 1.87 \pm 0.06 mmol.m⁻².s⁻¹ at low VPD level. In the plants subjected to 50 mmol NaCl of the variety CIP188002.1, the highest transpiration was observed on day 10

followed by day 2, day 4, day 8, and day 6 at high VPD and day 2 followed by day 4, day 10, day 6 and day 8 at low VPD level, respectively. The transpiration on day 10 and day 6 at high VPD level was 9.88 \pm 0.33 mmol.m⁻².s⁻¹ and 7.12 \pm 0.33 mmol.m⁻².s⁻¹, respectively. The transpiration on day 2 and day 8 at low VPD level was 5.51 \pm 0.61 mmol.m⁻².s⁻¹ and 2.99 \pm 0.06 mmol.m⁻².s⁻¹, respectively. The plants subjected to 50 mmol NaCl of variety CIP188002.1 had shown higher transpiration than the plants subjected to 0 mmol NaCl at both high and low VPD levels. The highest transpiration in plants subjected to 50 mmol NaCl at high and low VPD levels was 9.88 \pm 0.33 mmol.m⁻².s⁻¹ and 5.51 \pm 0.61 mmol.m⁻².s⁻¹, respectively. In case of plants subjected to 0 mmol NaCl, it was 7.41 \pm 0.29 and 3.28 \pm 0.31 mmol.m⁻².s⁻¹, respectively.

In variety CIP189151.8, the highest transpiration in the plants subjected to 0 mmol NaCl was observed on day 2 followed by day 6, day 8, day 10, and day 4, respectively at high VPD levels. In the case of low VPD levels, the highest transpiration was on day 2, day 6, day 10, day 4, and day 8, respectively. The transpiration on day 2 at high and low VPD levels was 7.48 ± 0.31 mmol.m⁻².s⁻¹ and 3.26 ± 0.33 mmol.m⁻².s⁻¹. The transpiration on day 4 at high VPD level was 4.53 ± 0.10 mmol.m⁻².s⁻¹ and on day 8 at low VPD level was 1.68 ± 0.10 mmol.m⁻².s⁻¹. The highest transpiration in plants subjected to 50 mmol NaCl of the variety CIP189151.8 was observed on day 10 followed by ay 4, day 8, day 6, and day 2 at high VPD level, respectively, and day 6 followed by day 10, day 4, day 2 and day 8 at low VPD level, respectively. Transpiration on day 10 at high VPD level was 8.30 ± 0.17 mmol.m⁻².s⁻¹. The lowest transpiration day with 5.58 ± 0.28 mmol.m⁻².s⁻¹ at high VPD level was on day 2. Other than day 2, the transpiration on the remaining days was close to each other. Transpiration on day 6 and day 8 at low VPD level was 3.69 ± 0.25 mmol.m⁻².s⁻¹ and 1.79 ± 0.15 mmol.m⁻².s⁻¹, respectively. Plants subjected to 0 and 50 mmol NaCl of the variety CIP189151.8 had shown no difference in the amount of transpiration. But there was a difference in the order of higher transpiration.

Plants subjected to 0 mmol NaCl of both the varieties had similarities in the amount of transpiration and the order of higher transpiration. The order of higher transpiration in both the varieties at high and low VPD levels was day 2>day 6>day 10>day 8>day 4 (day 4>day 8 for CIP189151.8) and day 2>day 6>day 8>day 10>day 4. But in the plants subjected to 50 mmol NaCl, variety CIP188002.1 had higher transpiration than CIP189151.8. The highest transpiration of the variety CIP188002.1 at high and low VPD level was 9.88 ± 0.33 mmol.m⁻².s⁻¹ and 5.51 ± 0.61 mmol.m⁻².s⁻¹, respectively. In case of CIP189151.8, the highest transpiration was 8.30 ± 0.17 mmol.m⁻².s⁻¹ and 3.69 ± 0.25 mmol.m⁻².s⁻¹, respectively.

In a comparison of the plants grown in 40% and 80% rH conditions, plants grown in 40% rH conditions had higher transpiration at both high and low VPD levels in the VPD chamber. Highest transpiration in the plants grown in 40% rH conditions was 12.47 ± 0.64 mmol.m⁻².s⁻¹ and in 80% rH conditions was 9.88 ± 0.33 mmol.m⁻².s⁻¹. There was a difference in the order of higher transpiration days in both 40% and 80% rH conditions.

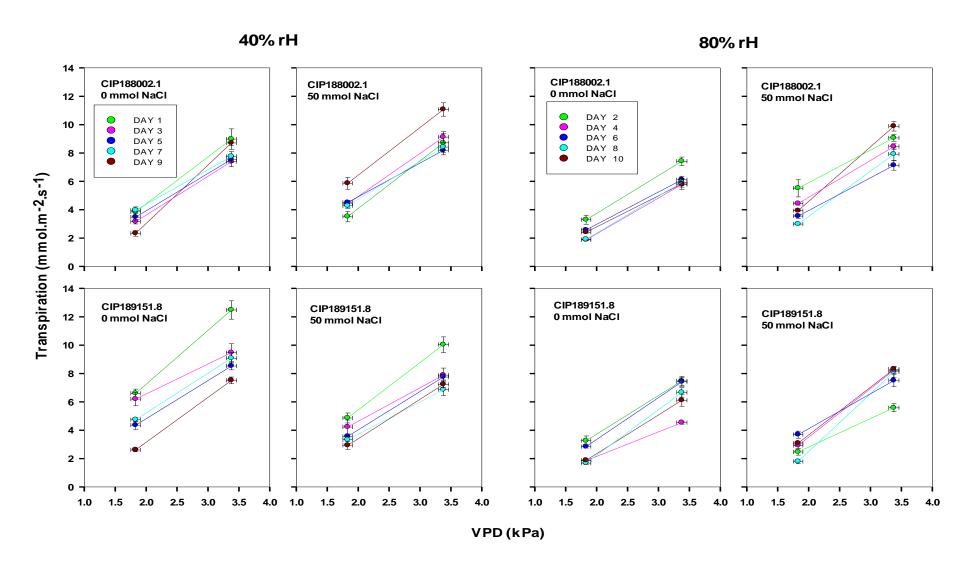


Figure 9: Response of plants in terms of transpiration for the sudden change of VPD level (Measurements for 40% rH and 80 % rH conditions started with low VPD and high VPD, respectively).

4.7 Acclimation effect:

Another interesting effect observed in this process of measurement of transpiration was the acclimation effect. Plants got to adapt to the environmental conditions in the measuring chamber over the days of measuring transpiration. The slope of regression lines in Figures 8 and 9 had become less and less steep with every repeat measurement from day 1 to the final day of the measurement. The slope of the regression lines at the highest VPD level (3.31 kPa) from day 2 to day 10 were plotted as the graph shown in Figure 10. For variety CIP188002.1, plants without any treatment had clearly shown a steeper line in both 40% and 80% rH conditions, and plants treated with 50mmol NaCl had negative slope value on day 3 and day 7 in 40% rH conditions, Slope was decreased on day 6 and then increased on day 8 and 10 in 80% rH conditions. For variety CIP188002.1, plants with no treatment had a steeper curve from day 3 to day 7, and the curve moved downwards on day 9. Plants treated with 50 mmol NaCl had shown a perfect increase in slope without any steepness. For variety CIP189151.8, plants treated with 0 and 50 mmol NaCl had a similar trend in the slope. An increase from day 4 to 6, then decrease on day 8 and finally raise on day 10. After observing all these curves, except the plants treated with 50 mmol NaCl of variety CIP188002.1 in 40% rH conditions, the remaining plants got to acclimate to the measuring conditions in the chamber. This leads to a change in the behaviour of the plants. This implies a learning effect. This effect might increase linearly with time or a more drastic level shift after a certain time. To avoid this effect, there is a need to use new plants every time to take measurements at a later stage.

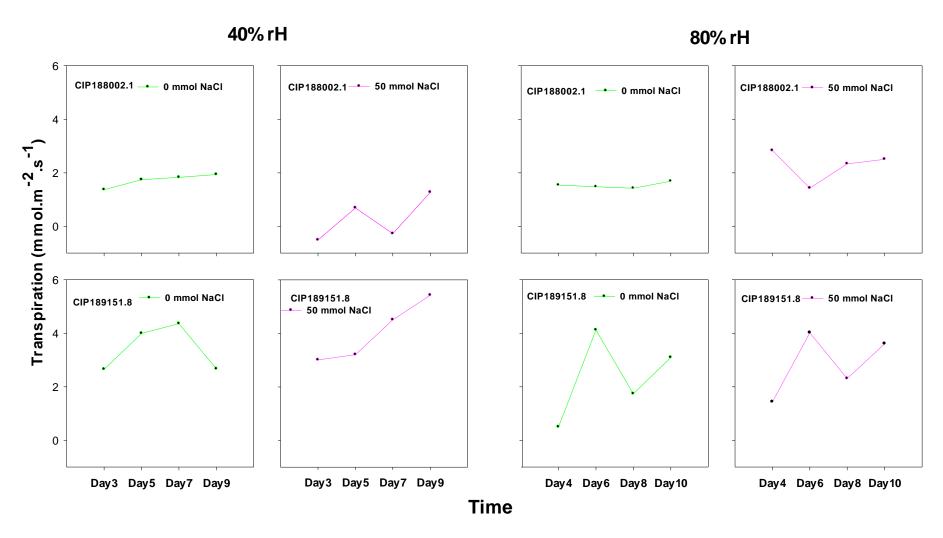


Figure 10: Increase of the sensitivity of the stomata changes over the time

5. Discussion:

5.1 Salinity and VPD on leaf area development:

5.1.1 Effect of salinity:

The plants were treated with 50mmol of NaCl on the 19th day of planting. The leaf area was measured every day till the leaf gets fully developed. There was a difference in the leaf area between the two varieties because of the difference in phenotypical characters of the varieties. The variety CIP188002.1 has bigger leaves than CIP189151.8. The variety CIP188002.1 can tolerate the salinity compared to the other variety CIP189151.8.

Leaf area is a good indicator of salt stress, since the leaf expansion needs high turgor pressure for enlargement of the cell, causing a direct effect on growth and photosynthesis (Sunil et al., 2014) Though there was no significant difference between the plants subjected to 0 mmol NaCl and salt, the leaf area of plants subjected to 50 mmol NaCl was reduced slightly than plants subjected to 0 mmol NaCl in the variety CIP188002.1, in the dry conditions. But in the case of wet conditions, in the same variety, the plants subjected to 50 mmol NaCl have crossed the plants subjected to 0 mmol NaCl in the leaf area. This variety has shown tolerance to the salt mainly in wet conditions. The other variety CIP189151.8 in wet conditions showed a reduction in leaf area in plants subjected to 50 mmol NaCl over plants subjected to 0 mmol NaCl. Generally, the reduction of the leaf's expansive growth is the first effect caused by salt stress (Curtis & Läuchli, 1987). It was already reported in sweet potatoes (Jafri & Ahmad, 1995). The salinity stops the cell division and expansion which leads to reduction of leaf area (Tanveer et al., 2020). The leaf area reduction in different cultivars of sweet potato under 14mM NaCl was also observed by Rodríguez-Delfín, Posadas, and Quiroz (Rodríguez-Delfín et al., 2014). The leaf area was reduced by 28% with the increase of the salinity from low to high. This may result in lesser photosynthetic activity (Rodrigues et al., 2021), but in dry conditions, both have similar leaf areas. There were also some contrasting findings of the reduction of the leaf area by the salt stress. There was no significant difference was found in the leaf size of the soybean among 0, 17, and 50 mmol NaCl (Kao et al., 2006). There was very little effect on the leaf development after the salt treatment with 50mmol of NaCl (Yeo et al., 1991). This also happened in the peas with 50mmol NaCl (Delgado et al., 1994). According to Mukherjee, some of the genotypes were not showed any negative effect up to 0.5g/l of NaCl. But he observed the reduction of leaf number in salt plants over control plants at 0.5g/I NaCl (Mukherjee, 2001). Here in our experiment, the leaf area was focused mainly. The leaf number was not affected by the salt, both plants subjected to 0 and 50 mmol NaCl had the same leaf number in both varieties.

In the case of variety CIP188002.1, there was a slight delay in leaf development in plants subjected to 50 mmol NaCl (29 days) than plants subjected to 0 mmol NaCl (25 days) in both dry and wet conditions. In the variety CIP189151.8, no delay in leaf development was observed. The leaf development took 28 days in both plants subjected to 0 and 50 mmol NaCl in 80% rH conditions. But in 40% rH conditions, plants subjected to 0 and 50 mmol NaCl took 27 and 25 days, respectively. A decrease in leaf expansion is the first effect observed by the plants exposed to salt. This leads to a delay in the leaf development. Plants subjected to 50 mmol NaCl had shown delay in the leaf development than plants subjected to 0 mmol NaCl in cotton crop (Jafri & Ahmad, 1995). An external osmotic potential caused by salt stress reduces the water uptake and affects leaf development (Tanveer et al., 2020). Though there was no significant reduction in leaf area, a few older leaves of the variety CIP189151.8 were dried and then died at the end of the experiment. Due to salinity, plants lose the ability to absorb the water which results in the reduction of plants growth. A high level of salt can lead to leaf injury and death. This happens when the salt builds up heavily in the cytoplasm (de Oliveira et al., 2013). Salt stress affects plant growth by leaf burn and leaf chlorosis (Kitayama et al., 2020). In this experiment, the plants were exposed to salt for 13 days which was a short period. The leaf toxicity and leaf falling were observed in sweet potatoes after the 21 days of 50 mmol of NaCl treatment (Kitayama et al., 2020). The effects of salt stress on the plants in short term are impermanent. But in the long term plants can get affected severely and die (Yeo et al., 1991).

5.1.2 Effect of VPD:

Humidity can be expressed as Vapour Pressure Deficit. It has different effects on leaf development (Mulholland et al., 2001). In this experiment, the plants were grown in two different growth chambers with 40% and 80% humidity levels. The leaf area differed between the plants grown under high humidity (80% RH) and low humidity (40% RH). Plants grown in wet conditions had a larger leaf area than dry conditions. The rice plants grown in high RH have reported a larger leaf area than the lower rH (Hirai et al., 2000). There were already some findings in lettuce, sugar beet, and wheat that the largest leaves were produced at higher relative humidity (Mortensen, 1986). The leaf number of the plants subjected to 0 and 50 mmol NaCl of both varieties was similar in both the 40% and 80% RH chambers. There was a finding in the chrysanthemum, the leaf surface area was higher under high humidity when there was an equal leaf number in both conditions (Codarin et al., 2006). This also differed between the variety and treatment. The variety CIP188002.1 has a higher leaf area in wet conditions for both plants subjected to 0 and 50 mmol NaCl. The leaf expansion was increased under high rH conditions in tomato and cucumber. The increase in leaf area was mainly because of the developed water relations and change in the cell wall properties like reduction of yield threshold and improvement in plastic extensibility.

The other reason for the increase of the leaf area was the very quick photosynthesis process in a very short period due to the higher stomatal conductance under high RH (Mulholland et al., 2001). For CIP189151.8, plants subjected to 0 mmol NaCl had a higher leaf area, than 50 mmol NaCl in dry conditions. For CIP188002.1 plants subjected to 50 mmol had higher leaf area than the plants subjected to 0 mmol NaCl in 80% rH conditions. Humid conditions even favoured the plants subjected to 50 mmol NaCl. An increase in the leaf area was temporary. Though the leaf area was increased for the first four weeks, then it was decreased under high humidity in tomatoes. The decrease of leaf area can be due to high turgor under high humidity. The leaf area can be restricted with long-duration exposure of plants with high humidity (Mulholland et al., 2001).

Though the plants subjected to 50 mmol NaCl of the variety CIP189151.8 in 40% rH conditions showed larger leaf area than the plants subjected to 50 mmol NaCl of 80% rH conditions, the plants subjected to 0 mmol NaCl of CIP189151.8, and both plants were subjected to 0 and 50 mmol NaCl of the variety CIP188002.1 had smaller leaf area than the plants grown in 80% rH conditions. High VPD harms the leaf area (Jyostna Devi et al., 2015). It causes a lower leaf area (Liu et al., 2006). The reduction of leaf area under high VPD was related to the decrease of the leaf expansion (Jyostna Devi et al., 2015). This leads to the reduction of the whole plant light interception area and photosynthesis per unit area which ultimately reduces the plant growth (Shibuya et al., 2018). The decrease of the leaf area suggests an adaptation that helps the plants in the regulation of higher leaf temperatures by maintaining a high transpiration rate in the hot dry regions (Liu et al., 2006). There was also a contrasting finding, the potato cultivars had higher tuber yield at a high rH (85% rH) level. But the leaf area was higher at a low rH level (50%) (Wheeler & Tibbitts, 1989).

5.1.3 Salinity and VPD interaction:

The interaction of VPD and salinity has a positive response on salt-tolerant variety, CIP188002.1 under low VPD. Some reports state that the RH has no enhancing effect on NaCl-induced growth reduction in the cotton and wheat plants. Some contrast findings state that the increasing air humidity can improve the NaCl-induced growth reduction in beans, barley, and tomato (An et al., 2005). The salt-sensitive variety, CIP189151.8 has performed better in the high VPD conditions than the low VPD conditions. The tolerance for NaCl increased under high transpiration conditions in *Phragmites australis*. But, in soybean, the salt-sensitive varieties have better growth under high humidity but no effect on salt-tolerant variety (An et al., 2005).

There was no significant difference in the dry weight and vine length between the plants subjected to 0 and 50 mmol NaCl of the variety CIP188002.1 in both 40% and 80% rH conditions. According to the previous studies, the increase in the VPD decreases the dry weight (Ray et al., 2002).

But here the plants grown under high VPD conditions have higher dry weight than the plants grown under low VPD conditions. In the case of variety CIP189151.8, which is sensitive to salt stress, the dry weight of the plants subjected to 50 mmol NaCl was lower than plants subjected to 0 mmol NaCl. Salinity had a significant effect on this variety. The previous findings have supported this result. The dry weight under salt stress was reduced significantly in the rice crop. The NaCl toxicity and improper nutrient uptake are the main reasons for the reduction of the dry weight under salinity (Puvanitha & Mahendran, 2017).

5.2 Salinity and VPD on Transpiration:

To know the effect of salinity and VPD on transpiration, parameters like cumulative water loss (g), transpiration rate (mmol.m⁻².s⁻¹) were calculated from the data of daily water loss by the plants in the two chambers.

The overall transpiration by the plants was higher in the 40% RH chamber than in the 80% RH chamber. The cumulative water loss in the 80% RH chamber was nearly half of the 40% RH chamber. The salt has not shown any significant effect on transpiration in both chambers. There was no considerable difference in the overall amount of water transpired by both plants subjected to 0 and 50 mmol NaCl in both CIP188002.1 and CIP189151.8 varieties. According to the previous findings, in the initial period of salt-action, there was low transpiration and stomatal conductivity. This results in a higher tolerance to salt stress in the form of improved extension growth and less accumulation of toxic ions (de Oliveira et al., 2013). According to Nishida et al., there was a significant decrease of cumulative transpiration by the salt treatment (Nishida et al., 2009). In the case of transpiration rate, there was a significant difference between the plants subjected to 0 and 50 mmol NaCl of the variety CIP189151.8 in the 80% RH chamber. The plants subjected to 0 mmol NaCl had a higher transpiration rate than the plants subjected to 50 mmol NaCl. The salt has shown its influence on the reduction of transpiration. But, in the other variety CIP188002.1, there was no difference observed between the plants subjected to 0 mmol and 50 NaCl and salt. There was also no difference observed in CIP189151.8 under 80% rH conditions. The general theory about salinity is, it can influence transpiration. This influence could be positive or negative. There were already several reports that the salinity can increase and decrease transpiration. According to BRAG 1972, the mix of Na⁺ and KCI has increased the transpiration by 50% in wheat and pea (Brag, 1972). There were also some contrasting findings to the previous records which state that the transpiration rate was reduced with increasing salinity level (Mert et al., 1976) (Ashby & Beadle, 1957) because of lesser soil water availability (Stewart et al., 1977). Though there was no significant difference, the overall transpiration in the plants subjected to 50 mml NaCl was slightly lower than the plants subjected to 0 mmol NaCl in the variety CIP188002.1 in 40% rH conditions. An increase in salinity level can reduce the osmotic potential in the soil which results in reduced water uptake and less transpiration due to stomatal closure (Ben-Asher et al., 2006).

Accumulation of abscisic acid can cause stomatal closure (de Oliveira et al., 2013). This results in the reduction of the photosynthetic parameters of the plant leaves (Wang et al., 2021).

Plants subjected to 50 mmol NaCl had lesser transpiration than the plants subjected to 0 mmol NaCl at a similar leaf area in both varieties under 40% RH conditions. Leaf area was almost similar in both plants subjected to 0 mmol NaCl and salt for the last six days in both varieties. But there was a considerable difference in the amount of water transpired by the plants subjected to 0 and 50 mmol NaCl. In both the varieties plants subjected to 0 mmol, NaCl had higher transpiration than 50 mmol NaCl at a similar leaf area. Transpiration rate was significantly reduced in plants subjected to 50 mmol NaCl in the plants of *Populus tomentosa* (Chen et al., 2003). The hydraulic conductivity of the plant roots can be reduced by salt stress. This results in a decrease in the water uptake by the roots. (Azevedo Neto et al., 2004). Under 80% RH conditions, variety CIP188002.1 had similar leaf area and transpiration in both plants subjected to 0 mmol NaCl and plants subjected to 50 mmol NaCl. But in the variety CIP189151.8, the plants subjected to 50 mmol NaCl had lesser leaf area. So, lower transpiration than the plants subjected to 0 mmol NaCl. The transpiration in the wheat was lower in plants subjected to 50 mmol NaCl due to a reduction of leaf area and stomatal closure (Nishida et al., 2009).

5.3 VPD and Transpiration:

The effect of different VPD levels on transpiration was tested for five alternate days in the Transpiration Measuring chamber. The four VPD levels were 3.31 kPa, 2.77 kPa, 2.18 kPa, and 1.70 kPa. The Plants were also given sudden VPD shock, where they were given two contrasting VPD levels within no time, 3.37 kPa to 1.82 kPa and vice versa.

Plants grown in 40% rH conditions had higher transpiration than 80% rH conditions at four different VPD levels. There was no significant difference between plants subjected to 0 mmol NaCl and salt. This might be due to less NaCl concentration, 50mmol in the experiment. According to previous findings, salt stress can reduce transpiration. Salt stress had limited the transpiration in the *Tamarix aphylla* plants (Hagemeyer, 1989). Reduction in the stomatal conductance can cause lower transpiration (Ray et al., 2002). The difference in transpiration between the varieties was observed in this experiment. The variety CIP189151.8, sensitive to the salt stress had transpired more water than the other variety CIP188002.1, tolerant to salt stress. This happened in both 40% and 80% rH conditions. This was also observed in the previous experiments. The salt accumulation and transpiration were higher in CIP189151.8 than in the variety CIP188002.1 (Schopfhauser, 2020). High transpiration due to high VPD

can be restricted by expressing stomata closure. This is to limit the flow of water from the plant roots to the transpiration sites of leaf surfaces (Yang et al., 2012). This stomatal closure causes a reduction in photosynthesis (Grossiord et al., 2020).

Under the 40% RH conditions, the transpiration was increased with increasing VPD levels. This was seen in both plants subjected to 0 and 50 mmol NaCl of both varieties. The transpiration mainly depends on the vapor pressure deficit, leaf area, and solar radiation (Leonardi et al., 2000). There were several reports that transpiration and VPD are positively correlated. In maize, an increase in VPD levels, increased the overall transpiration (Ray et al., 2002). This can happen till a certain point of VPD when the transpiration starts to decline or remains high (Grossiord et al., 2020). The plants may wilt or sometimes die due to higher transpiration because of higher VPD (Chiango et al., 2021). In the 80% RH chamber, the transpiration was increased with increasing VPD level, but this happened from the second VPD level. At the first VPD level, 1.66 kPa, the transpiration was higher than the second VPD level, 2.18 kPa. This happened in both plants subjected to 0 and 50 mmol NaCl of both varieties. The transpiration was higher at all VPD levels for the plants grown under 40% RH compared to the plants grown under 80 RH conditions. Generally, the transpiration rate is low under low VPD conditions. This is due to high air humidity which does not absorb any more water from the plants. In case of higher VPD conditions, the plant transpires more because the air humidity is low, so the dry air absorbs water from the plants. This happens till the air gets saturated. Later, the air humidity increases then the transpiration gets reduced (Nederhoff & Houter, 2009). Though there was no proper order for the highest transpiring days, the highest transpiration was observed mostly on the first day of the measurements. This was commonly observed under 40% and 80% RH conditions. But there was a contrasting finding to this in maize that the transpiration was lower on the first day than the second day in the maize crop at different VPD levels (Yang et al., 2012). But there was an exception of plants subjected to 50 mmol NaCl of the variety CIP189151.8 in 40% rH conditions. The highest transpiration was observed on the 7^{th} day of the measurements. The lowest transpiration of plants in 40% rH conditions was observed on the last day of measurements (9th day) in both the varieties except for plants subjected to 0 mmol NaCl of variety CIP189151.8, where lowest transpiration was on 7th day of measurements. The lowest transpiration on the last day could be due to damage of few leaves due to salt stress and due to the partial closure of stomata. The stomata closure can affect the plant growth. To avoid this, under high VPD conditions, transpiration should happen to certain level only. After that level, transpiration under high VPD results in higher loss of water from the plants. The roots sometimes cannot replace the water losing through transpiration which leads to the wilting of the plant and ultimately death of the plant (Nederhoff & Houter, 2009). The plants grown in 80% rH conditions had shown different behaviour for the lowest transpiration day. Though the plants subjected to 0 and 50 mmol NaCl of variety CIP188002.1 had shown the same trend in the days of the highest order of transpiration, the variety CIP189151.8 had a difference in the order of transpiration.

Transpiration of plants under the sudden shock of two contrasting VPD levels was also observed. There was no significant change observed between plants subjected to 0 mmol NaCl and salt except under 80% rH conditions, the plants subjected to 50 mmol NaCl of the variety CIP188002.1 had higher transpiration compared to its transpiration on normal measurement days. Plants grown under 40% rH conditions had shown higher transpiration at two VPD levels than the plants grown under 80% RH conditions. The highest transpiration at a higher VPD level (3.37 kPa) was observed mostly on the last day of the measurement in the plants subjected to 50 mmol NaCl except for variety CIP189151.8 under 40% rH condition. The highest transpiration at lower VPD level (1.82 kPa) was observed mostly on the first day of the measurements except for the variety CIP188002.1 in 40% rH and plants subjected to 50 mmol NaCl of the variety CIP189151.8 under 80% RH condition. Generally, the transpiration rate increases with the increasing age of the plant. But this happens till a certain point, after that the transpiration doesn't change with the age of the plant (Röll et al., 2015).

7. Conclusions:

The 50mMol NaCl has shown no significant effect on plant development and transpiration. Though the leaf area differed between the two varieties, it was due to differences in the varietal phenotypical characters. Salinity had no significant effect on leaf area in both 40% and 80% rH conditions. Transpiration was increased with increasing VPD levels. There was no significant difference in the transpiration rate of both plants subjected to 0 mmol NaCl and plants subjected to 50 mmol NaCl. The total amount of water transpired by the plants subjected to 50 mmol NaCl and plants subjected to 0 mmol NaCl was also similar in both the VPD conditions. Both varieties had no significant difference in terms of transpiration rate, but they had a difference in plant dry weight and vine length. Total plant dry weight was reduced under salt treatment in the variety CIP189151.8 but no salinity effect in the CIP188002.1 variety. This is because the variety CIP188002.1 is tolerant to salt stress and another variety is sensitive to salt stress. The sudden change in the VPD to the contrasting level also did not show any difference in the amount of transpiration. The transpiration trend was like normal VPD treatment.

The plants of the variety CIP189151.8 had some drying symptoms at the end of the experiment. Few leaves were dried and fallen. This might be due to the sensitivity of the variety to the salt. This was not seen in the CIP188002.1 variety. The experiment was very short with only a one-month growing period, the salt stress was not observed. The salt concentration was also very low, 50 mmol. The replications were only four. It would be better to have a greater number of replications. So, more research needs to be done with longer periods, more replications, and with different salt concentrations to know the exact effect of salt on plant growth and its activities.

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