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IMPACT OF NACL, KCL, MCL_2, $MGSO_4$ AND $CACL_2$ ON THE LEAF ANATOMY OF CUCUMBER (*CUCUMIS SATIVUS CV.* MTI2)

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Abstract

This study was conducted to compare the effects of NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ on the anatomical of cotyledon leaves. Five types of salts (NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$) at different concentrations (50, 100, 150 and 200 mM) and deionized water as control are used. A 10 sterilized seeds were placed in Petri dishes containing 5 ml of deionized water or each salinity solution and kept in the growth chamber at 25 ± 1°C. On day 8th, coteyledon leaves were fixed for anatomical study. Results show the response of MTi2 on five different salts is significantly different. The degree histological changes of MTi2 cotyledon leaves increased as the concentration of salts increased. Salts significantly change the structure and arrangement of the upper epidermis cells, mesophyll tissue and lower epidermis cells, also reduce of intracellular space among the mesophyll cells. Results found that no anatomical changes of MTi2 cotyledon leave in any concentration of KCl.

Key words: *Cucumis sativus* cv. MTi2, salinity, cotyledon, histological.

Introduction

Salinity is a worldwide problem and one of the major abiotic stress, which causes a substantial reduction in crop yields particularly in arid and semi-arid regions with naturally high soil salt and low rainfall that inhibits leaching (Zhao *et al*., 2007). Physiological and metabolic processes are negatively influenced by soil salinity, which leads to decreased plant growth and yield (Azipour *et al*., 2010). Salinity is the presence of excessive concentrations of soluble salts in the soil that suppress plant growth. The major cations contributing to salinity are Na^+, Ca^{+2}, Mg^{+2}, K^+ and anions are Cl^-, SO_4^{-2}, HCO_3^-, CO_3^{-2} and NO_3^-. However, Na^+ and Cl^- ions are considered the most important (Wallender *et al.*, 2011) since Na+ in particular causes deterioration of the physical structure of the soil and both Na^+ and Cl^- are toxic to plants (Hasegawa *et al*., 2000). *Cucumis sativus* is a widely cultivated plant in the gourd family Cucurbitaceae. Most species of *Cucumis* have a South Asia origin (Renner *et al*., 2007), but now it grows on most continents The history of cucumber cultivation goes back 3,000 years when it was widely grown as it is today (Wehner and Guner, 2004). This plant is an important greenhouse crop in semi-arid areas with saline groundwater so it is necessary more research on the effect of salinity on the anatomy of this plant (Sato *et al*., 2006). Both the water and osmotic potentials of plants are more negative when salinity increase while turgor pressure also increases with higher salinity (Parida and Das, 2005; Mohammed and Nulit a; Mohammed and Nulit b, 2019). Plants adapt themselves in the saline atmosphere by altering their morphology during their growth under long term salinity exposure (Vinod *et al*., 2013). Changing the morphology leads the anatomical changes (Romero-Aranda *et al*., 2001; Parida and Mittra, 2004). Therefore, the aim of this research is to investigate the effects of individual salts NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ at concentrations 50 mM, 100mM, 150 mM and 200 mM on the anatomical changes of *Cucumis sativus* cv. MTi2.

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Materials and Methods

Preparation of seedling's Cotyledon leaves

A study was conducted at the Tissue Culture Laboratory of the Dept. of Biology, Universiti Putra Malaysia, Selangor. Five types of salts (NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$) at four different concentrations (50, 100, 150 and 200 mM) and deionized water as a control was used. Sterilization of MT12 seeds was carried out according to method by Panuccio *et al*., 2014;

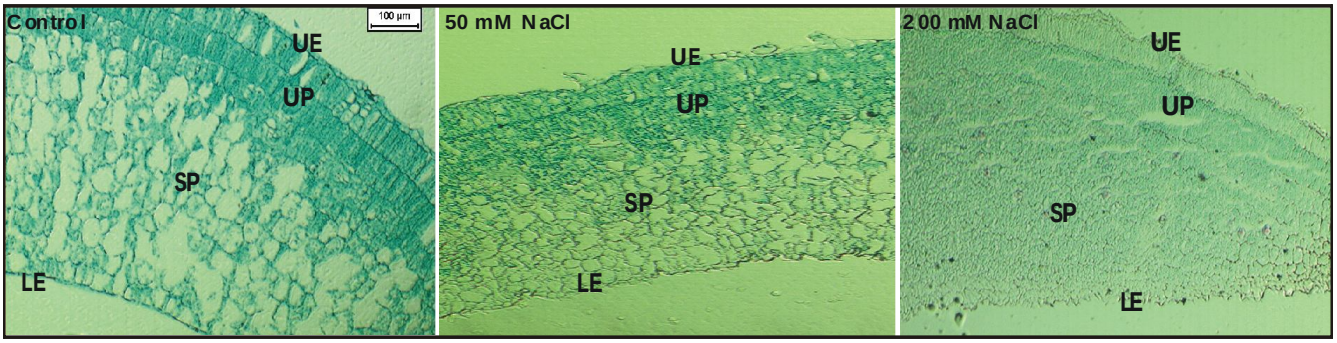

Fig. 1: Cross-section of MTi2 cotyledon leaves in control, 50 mM KCl, and 200 mM KCl. Magnification 50x. UE= Upper epidermis; UP=Upper palisade; SP=Spongy parenchyma; LE= Lower epidermis.

Mohammed and Nulit a; Mohammed and Nulit b, 2019). with slight modification. Mature, healthy and equal sized seeds were selected and were surface sterilized with 5% sodium hypochlorite for ten minutes. Subsequently, the seeds were rinsed with sterilized distilled water for three times and air-dried before being used in the germination tests to avoid any fungal attacks. A 10 seeds were placed on Whatman filter paper in sterilized petri dishes (9 cm diameter) containing with 5 ml of deionized water or each salinity solution. The petri dishes were hermetically sealed with parafilm to prevent evaporation and kept in the growth chamber at 25 ± 1°C. On day 8th, cotyledone leaves with same sizes were cut for anatomy study.

Preparation and fixation of samples MTi2

Seeds were germinated as described in subsection 1 Cotyledon leaves were cut immediately at day 8 and fixed in FAA (formaldehyde, acetic acid and 70% ethanol, 1:1:17 v/v) for 24 hours. Samples were washed under running tap water and stored in 70% alcohol (Chehegani *et al*., 2011).

• Dehydration of samples: Dehydration was conducted to remove intracellular liquid. This step was conducted by soaking samples in a series of ethanol 80%, 90%, 96% and absolute ethanol for 10 minutes in each phase.

• Clearing of samples: The intercellular spaces should be filled with toluene or xylene, which has the capacity to solve in melted paraffin. The samples were passed through the following solutions:

a. 3 volume absolute ethanol + 1 volume toluene for 10 minutes

b. Equal volumes of absolute ethanol and toluene for 10 minutes

c. 1 volume absolute ethanol + 3 volume toluene for 10 minutes

d. Pure toluene for 20 minutes.

• Embedding and sectioning of samples: Samples were embedded in the forming blocks for 24 hours and sectioned with Microtome (LEICA RM2235). The sections were fixed on the slides with warm plate and dried at 40-45°C for 24 hours.

• Staining and Mounting of samples: The sections were hydrated using a series of different concentrations of ethanol (100%, 96%, 90%, 70% and 50%) and washed for three minutes with distilled water after each hydration step. After hydration, the samples were stained using 1% safranin for 7 minutes and washed using tap water. Then, the samples were leached with 50% ethanol and then with 70% ethanol for 3 minutes. Afterwards, the samples were stained for 7 minutes in 1% fast green. Thereafter, dehydration was carried out gradually with increasing concentration of ethanol (90%, 96% and

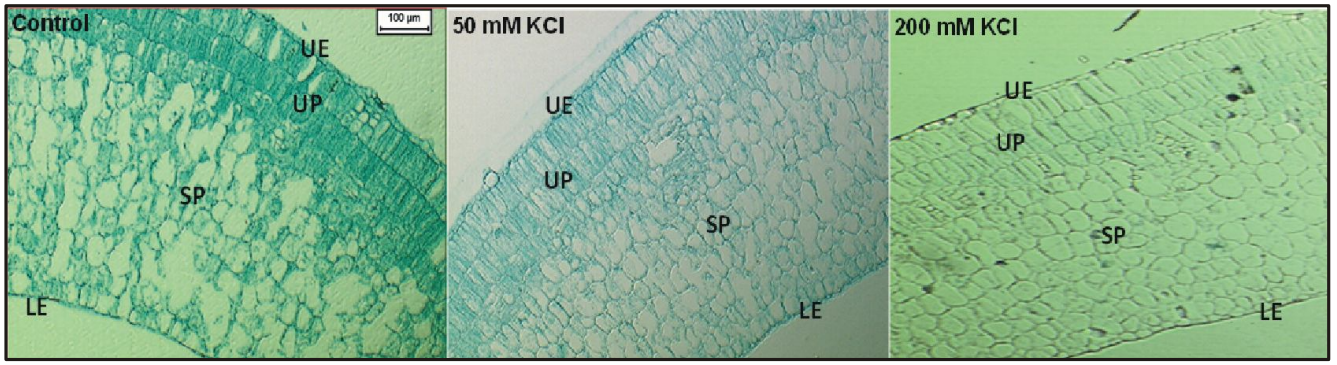

Fig 2: Cross-section of MTi2 cotyledon leaves in control, 50 mM KCl, and 200 mM KCl. Magnification 50x. UE= Upper epidermis; UP=Upper palisade; SP=Spongy parenchyma; LE= Lower epidermis.

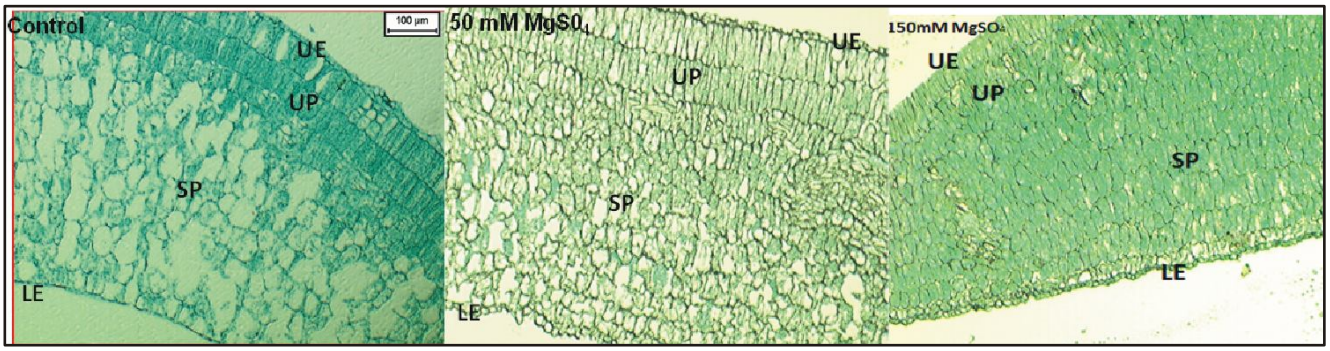

Fig. 3: Cross-section of MTi2 cotelydon leaves from control, 50 mM $MgSO_4$, and 150 mM $MgSO_4$. Magnification 50x. UE= Upper epidermis; UP=Upper palisade; SP=Spongy parenchyma; LE= Lower epidermis.

100%). Lastly, the samples were soaked in pure toluene for 3 minutes. Samples were mounted with covered using cover slips and observed under Leica light microscope.

Results

The anatomical changes of MTi2 cotyledon leaves highly affected by the type and concentration level of salts. Fig. 1 shows the cross section of the structure and arrangement of the upper epidermis cells, mesophyll tissues and lower epidermis cells slightly changed in 50 mM NaCl. As concentration NaCl higher, the degree structural changed to increase as shown in fig. 1. In addition, cotyledon tissues treated in higher concentration NaCl (200 mM) highly disrupted and destructive structural. On the other hand, KCl at any concentration do not effect on the anatomy of leaves of MTi2 cotyledon leaves as presented in fig. 2.

Fig. 3 shows the cross sections of MTi2 cotyledon leaves treated with different concentration of $MgSO_4$. The shape and arrangement of the mesophyll spongy and lower epidermis cells not highly affected in low concentration. But the shape and arrangement highly affected in high concentration $MgSO_4$ (150 mM). The layer of mesophyll palisade found reduced, condensation of mesophyll spongy cells and no intercellular space found.

The fig. 4 shows the anatomical changes of MTi2 seedling leaves was found not highly affected by $MgCl_2$. The size of leaf cells reduced in 50 mM $MgCl_2$ but the arrangement and shapes of lower epidermal cells and both mesophyll tissues disrupted in 100 mM $MgCl_2$. Also, no intercellular spaces in 50 and 100mM $MgCl_2$.

The fig. 5 shows the size of leaf cells reduced, mesophyll tissues condensed and no intercellular space in low and high concentration of $CaCl_2$

Discussion

Results revealed that each type of salt at different concentration had different effects on the histological changes of MTi2 cotyledon leaves. The degree of histological changes was also affected by the type and concentration of salts. A study of the cross sections of MTi2 cotyledon leaves revealed that the anatomical changes of cotyledon were highly affected by the concentration level of NaCl. There are significant changes in shape and arrangement of the upper epidermis cells, mesophyll tissue and lower epidermis cells in 50 mM NaCl. Higher concentration (200 mM) of NaCl, the level of changes increased, consequently, all types of cotyledon cells were unable to differentiate on each other. On the other hand, the anatomical changes of cotyledon leaves of MTi2 were slightly affected by the concentration level

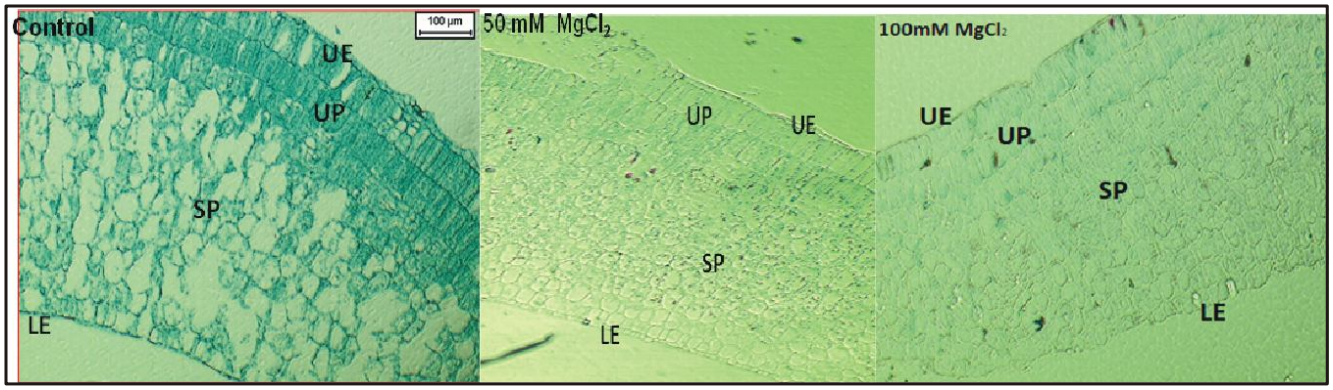

Fig. 4: Cross-section of MTi2 cotelydon leaves from control, 50 mM $MgCl_2$, and 100 mM $MgCl_2$. Magnification 50x. UE= Upper epidermis; UP=Upper palisade; SP=Spongy parenchyma; LE= Lower epidermis

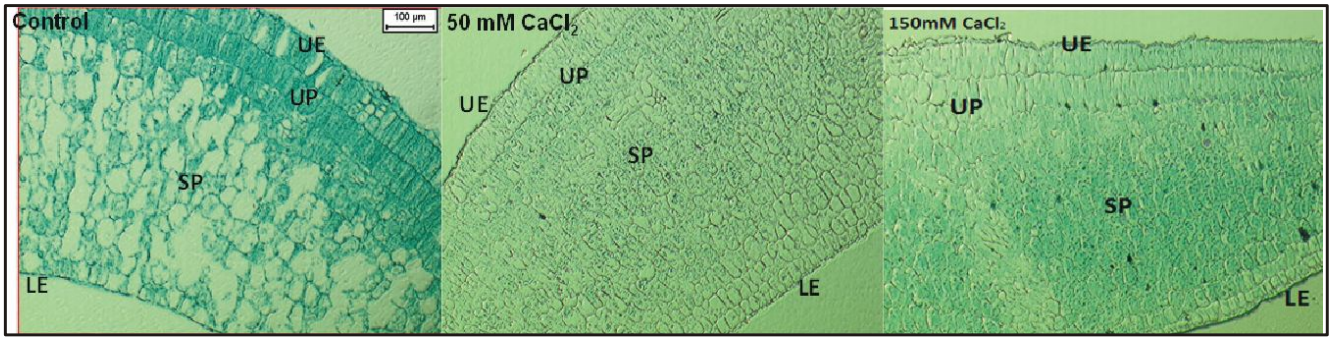

Fig. 5: Cross-section of MTi2 cotelydon leaves from control, 50 mM $CaCl_2$, and 150 mM $CaCl_2$. Magnification 50x. UE= Upper epidermis; UP=Upper palisade; SP=Spongy parenchyma; LE= Lower epidermis.

of KCl. The low concentration of KCl (50 mM) does not affect the anatomy of seedling cotyledons, but at high concentration (200 mM), mesophyll spongy was condensed and there were no intracellular spaces. At 50 mM $MgSO_4$, the shape and arrangement of the mesophyll spongy and lower epidermis cells were significantly changed. In high concentration (150 mM) $MgSO_4$, the second layer of mesophyll palisade was also affected (reduced), with mesophyll spongy condensed and no intercellular spaces. The anatomical changes of MTi2 cotyledon leaves were not highly affected by $MgCl_2$. The size of leaf cells was reduced in 50mM $MgCl_2$ but the arrangement and shape of lower epidermal cells and both mesophyll tissues were disrupted in 100 mM $MgCl_2$. Also, there was no intercellular space shown in 50 and 100 $MgCl_2$. The size of leaf cells was reduced, mesophyll tissues were condensed and there were no intercellular spaces in low and high concentrations of $CaCl_2$. Mansour, (1997); Tabaei-Aghdaei *et al.*, (2000); Jamil *et al.*, (2012) reported that the harmful impact of salinity on the plasma membrane is fundamentally because of the presence of salt ions which also causes water deficiency in plants and leads to the malfunctioning of cellular membranes. In addition, salinity caused a noticeable decline in all measurements of the wheat leaf lamina; leaf lamina thickness, thickness of upper and lower epidermis, thickness of lamina spongy ground tissue, main vascular bundle length and width, metaxylem vessel diameter as well as thickness of phloem and of fibrous bundle sheath (Shaaban, 2016). Salinity produces certain physiological, morphological and anatomical changes in cells (Isla *et al.*, 1998), or tissue and organ levels (Munns, 2002). Other anatomical modifications, for example, lack of differentiation, number and diameter of xylem vessels also occur in plants under salinity stress (Ola *et al.*, 2012). In general, plants grown in saline solution exhibited greater thickness in the cuticle, vascular tissues and vessels than unstressed plants while cortex zone thickness was reduced (Bijanzadeh and Kazemeini, 2014). The leaf cross-sectional area of barley during its development may be decreased under salt stress because of architectural changes of leaves. It can be concluded therefore that the decrease in the cross-section of barley is mostly the result of a smaller number of small veins. However, the decreased area of protoxylem and metaxylem in midrib and veins, especially at 25 mm from the leaf base may cause reduced growth under saline conditions.

Conclusion

Histological studies revealed that changes of MTi2 cotyledon leaves as a response to NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ are different. The concentration of salts also increased on the degree histological changes of MTi2 cotyledon leaves. However, other type of salts significantly changes the structural and arrangement of the upper epidermis cells, mesophyll tissue and lower epidermis cells, also reduce of intracellular space among the mesophyll cells. Results also found that no anatomical changes of MTi2 cotyledon leaves in any concentration of KCl.

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