

# Glutathione-Rich Nano Fertilizer Improved Parameters of Sweet Corn under Salinity Condition

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**Abstract:-** Salinity influenced the growth and yield of crops. Effects of GSH-rich nano fertilizer to reduce salinity stress remained unknown. The objectives of this study were to determine the effects of GSH-rich nano fertilizer on morphophysiological parameters of sweet corn plant and to compare the effectiveness between GSH-rich nano fertilizer and inorganic fertilizer. Seeds of sweet corn were grown in pots under different treatments of glutathione (GSH), sodium chloride (NaCl) and nano fertilizer. A Complete randomized design was used with three replicates. Treatments significantly affected morphophysiological parameters of corn plant ( $p < 0.05$ ). Treatment of nano fertilizer with GSH significantly increased morphophysiological parameters of sweet corn than that of plants treated without GSH with nano fertilizer under salinity condition. In addition, nano fertilizer showed significant results in increasing morphophysiological parameter over commercial inorganic fertilizer. This study indicated that GSH enhanced physiological functions corn plants under nano fertilizer to lessen salinity condition.

**Keywords—** Chlorophyll Content, Chlorophyll Fluorescence, Stomatal Aperture, Relative Water Content, Water Loss.

## I. INTRODUCTION

Salt stress causes necrotic leaf lesion in the minor vein due to formation of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> (Hernández et al., 2001 and 2002). The degree of formation of oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen increased as the concentration of salt increased and the stomata is closed by influences of CO<sub>2</sub> or O<sub>2</sub> go in and out of the stomata. In addition, increment of Na<sup>+</sup> in salt-treated plant decrease of leaf water content, leaf growth and leaf area (Hernández et al., 2002). Saline soils significantly reduce the viability of seedling and inhibited the growth of seedling leaves become yellow or pale green which indicate the delay in chlorophyll development (Roxas et al., 1997).

N-acetyl-cysteine (NAC) is a sister compound and by-product of glutathione (GSH) (Tausz et al., 2004 ; Kerkick & Willoughby, 2005) and antioxidant that minimizes oxidative stress. Externally application of NAC enters through the cell and increased chlorophyll content that is linked to the increment of GSH in cell (Jahan et al., 2014). GOS control reactive oxygen species (ROS) in guard cells (Jahan et al., 2008). Nano fertilizers are fertilizers which are modified through nano technologies where the shape and size at nano scale. The fertilizers are released gradually, increase the efficiency of fertilizer absorption by plant and soil. Therefore, reduce the loss of the fertilizers from

leaching out from the soil and increases availability of plant uptake (Rai et al., 2012). Other than that, the fertilizer application are decreased which reduced soil toxicity caused by over dosage (Naderi & Danesh-Shahraki, 2013).

Tausz et al. (2004) clarified that, GSH used as stress marker in plant. The concentration of GSH increased in initial of stress response, redox state oxidized then plant system degraded. Besides, GSH kept Reactive Oxygen Species (ROS) level in stress plant under control. For instance, stomata closed due to environmental stress and at the same time light exceed demand of photosynthesis process. Excess energy reduced electron transport chain, leads to the formation of ROS by univalent reduction of oxygen. GSH helped in dissipation of excess energy to harmless heat. Moreover, yield of Cd tolerant rice and Cd sensitive rice increased after applied exogenous GSH (Cao et al., 2015). Cao et al. (2015) further explained that the application of GSH coupled with low Cd accumulation reduced the Cd in grain grew in Cd polluted soils. Teh et al. (2015) found that exogenous application of GSH enhanced the plant height of rice (MR 220) and chlorophyll content of two types of rice (MR 220 and MR 253).

*Zea mays* L or sweet corn is a monocot belongs genus *Zea*, in the grass family Poaceae. It is an annual grass, consists of both female and male reproductive parts which contains high of Vitamin A and it is originated in Mexico. To date, it is widely planted in the world due to the grain. The growth stage is divided into two stages; vegetative (commonly take 60 days) and reproductive. Sweet corn gets its name from special genes that prevent or retard the normal conversion of sugar to starch during kernel development (Pennstate Extension, 2017). *Zea mays* is one from four plants that considered as glycophytes (salt sensitive plants) including rice, soy bean and bean (Mahajan & Tuteja, 2005). Kravchik and Bernstein (2013) stated that mature maize leaves cells are more sensitive to high salinity than younger cells which favour higher expression of genes of the antioxidant enzymes.

Tekeli and Kale (2017) discovered that, salinity inhibits the growth and reduced yield of sweet corn. Salinity conditions inhibit the growth and development of *Zea mays* by reducing cell division, cell enlargement and increasing the accumulation of hydrogen peroxide (Shtereva et al., 2015). On the other hand, biomass in root of maize treated with salt was reduced while the accumulation of NA<sup>+</sup>

activities of enzymatic antioxidant, ASC and GSH were increased in organ of plant like root, matured and young leaves (Abdelgawad et al., 2016). Farooq et al. (2015) investigated that, germination of maize was sensitive to salt stress. High levels of sodium and Cl in rhizosphere decreased the uptake of nutrients like calcium, nitrogen, potassium, magnesium, etc by plant roots.

## II. MATERIALS AND METHODS

Sandy soils and peat moss were mixed with a ratio of 3:1 before being added into the pot. Nano fertilizer, sodium chloride and glutathione were prepared and poured into the pot according to the treatments (Table 1). Based on the Table 1, eight treatments with three replicates were used in this study. Plants were grown in a plant growth room under 16 hours of light and 8 hours of dark cycle conditions including control temperature ( $22 \pm 2$  °C) and relative humidity ( $60 \pm 10$  %). After five weeks, morphophysiological parameters were recorded.

	G0F0	G1F0	G0F1	G1F1
N0	T1	T2	T3	T4
N1	T5	T6	T7	T8

Table 1 :- Combination of the Treatments in This Study

Salinity, N: N0-0 mM and 50 mM,  
 Nano fertilizer, F: F0-with nano fertilizer and F1-without nano fertilizer (inorganic fertilizer was used)  
 GSH, G: G0-0  $\mu$ M and G1-100  $\mu$ M.

### A. Measurement of parameters

#### ➤ Measurement of plant height:

The height of the plant was measured from the soil surface to the longest leaf emerged from the whorl by straighten the plant to its fullest length by using a ruler.

#### ➤ Measurement of root length:

The seedlings were uprooted then cleaned. The length of root was measured by using ruler. The length of the root was measured started from base of the stem until the end of the root.

#### ➤ Measurement of light dependent parameters:

Chlorophyll content was measured using a SPAD-502 portable chlorophyll meter from 11 am to 12 pm to avoid moisture on leaves (Chelah et al., 2011; Jahan et al., 2013). The SPAD-502 portable chlorophyll meter was calibrated before use. The sample was inserted into the sample slot of the measuring head. The measuring head was pressed on the finger rest and it was held closely until beep sounds. The measured value appeared in the display.

#### ➤ Measurement of chlorophyll fluorescence:

Chlorophyll fluorescence emission such as minimal fluorescence (Fo), maximal fluorescence (Fm) and quantum yield of PS II (Fv/Fm) from leaf disc or leaf attached to the plant was measured according to the guidelines of the JUNIORPAM fluorometer (Walz, Germany). Measurements

were made on upper (adaxial) surface of leaves (Hichem et al., 2009).

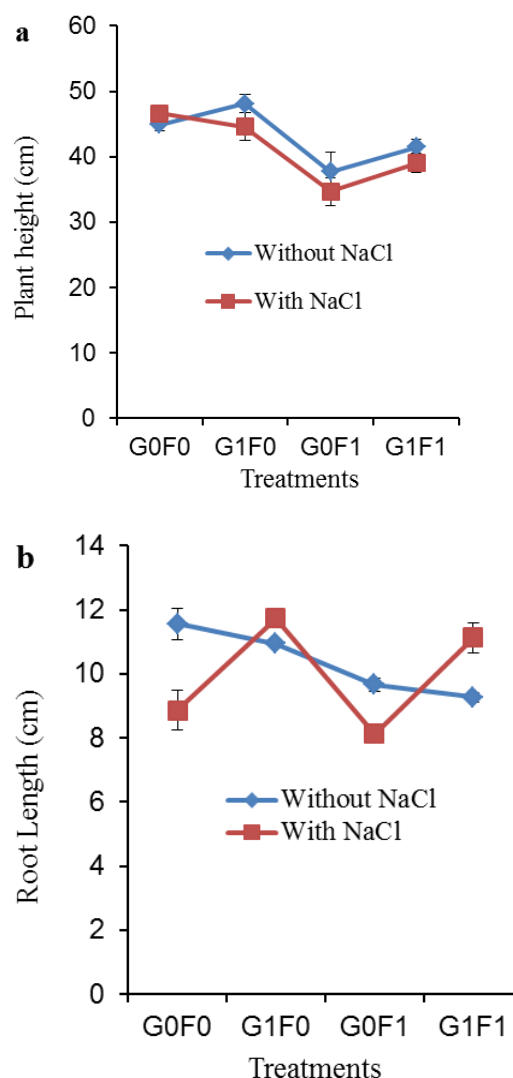
#### ➤ Measurement of stomatal aperture:

Stomatal assay was prepared as per previous method (Jahan et al., 2008, 2011). The leaf was cut and blended using a blender. The blended leaf was poured into nylon mesh filter. After that, nylon mesh filter was blotted using tissue paper. The epidermal peel was put on the slide and covered with coverslip. Images of stomata apertures were captured using an Olympus BX53F microscope connected to DP80 digital imaging colour camera and were analysed with image analysis software cellSens Dimension 1.9 (Olympus, Japan). The width of stomatal aperture was measured as previously described (Jahan et al., 2014).

### B. Statistical analysis

Data were analysed for analysis of variance (ANOVA). The means were compared using Duncan’s Multiple Range Test (DMRT) at 5 % by using the SPSS software version 17. P values < 0.05 were considered as statistically significant.

## III. RESULTS



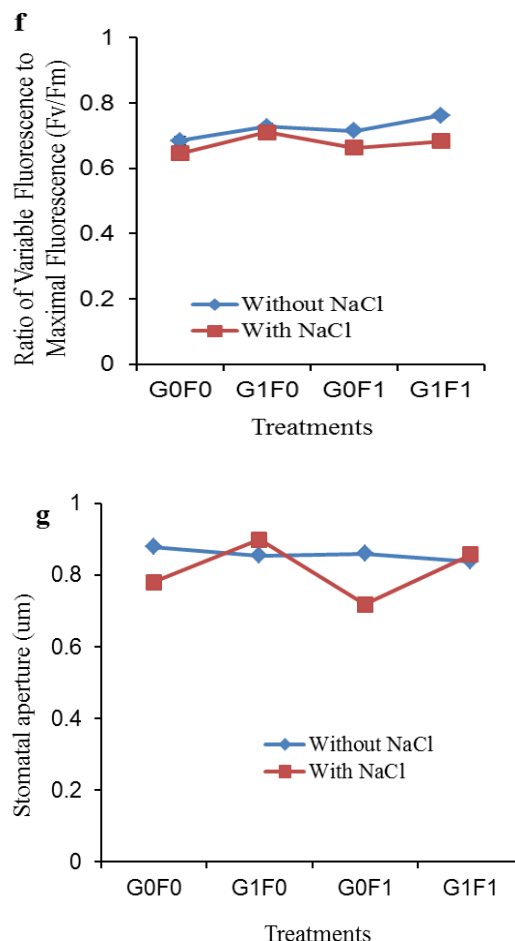
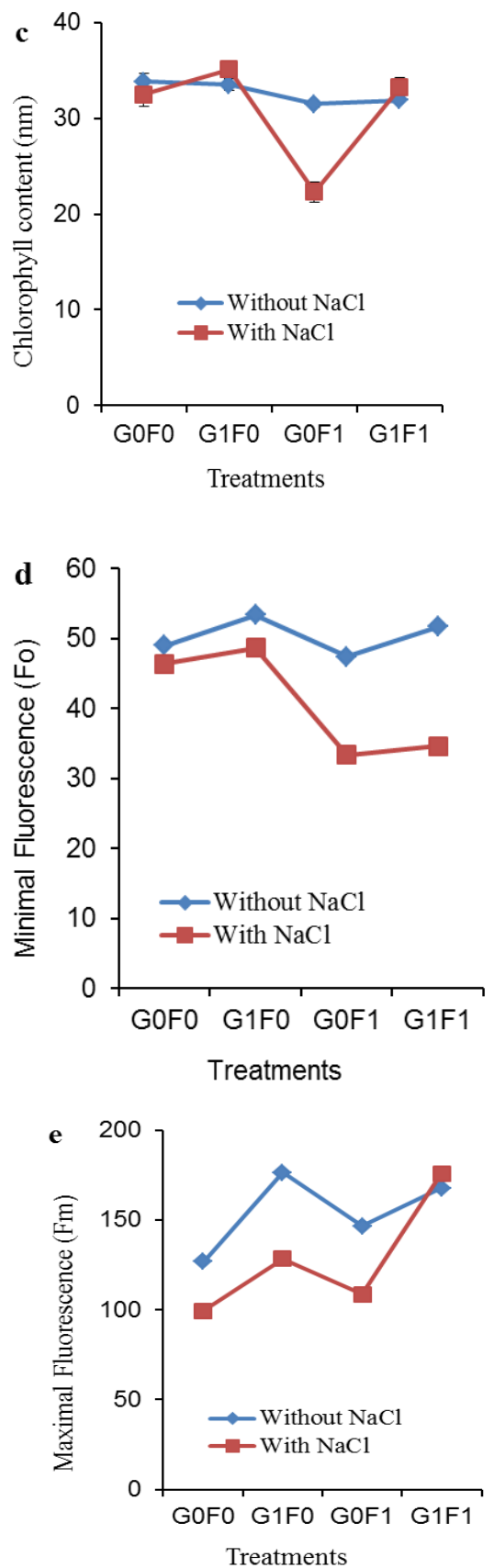


Fig 1 :- The effects of treatments on a)plant height, b)root length of corn, c)chlorophyll content, d)minimal fluorescence (Fo), e)maximal fluorescence (Fm), f)ratio of variable fluorescence to maximal fluorescence (Fv/Fm) and g)stomatal aperture.

**IV. DISCUSSION**

Plant height is a reliable parameter of growth and development, representing the build-up of cell after influenced by different treatments. Plant height of sweet corn with and without NaCl condition were measured and presented in figure a. Sweet corn under control condition (G0F0) showed almost similar plant height. Overall, plant height of sweet corn without NaCl was higher under different treatments compared to sweet corn with NaCl. Plant height of sweet corn under normal condition (without NaCl) was slightly increased with the application of GSH (G1F0). Then, it decreased after being applied nano fertilizer (G0F1) and increased again after application of GSH- rich nano fertilizer (G1F1). Plant height of sweet corn under saline condition (with NaCl) was slightly decreased with single application of GSH (G1F0) and nano fertilizer (G0F1). On the other hands, it was increased with the application of GSH-rich nano fertilizer.

Root is the organ in plant; give support to the plant body by anchoring to the ground, absorb nutrients and water. Figure b showed root length measured in centimetre versus treatments. In general, root length of sweet corn without saline condition was declined after being applied by different treatments where in saline condition, it showed fluctuations. Root length of sweet corn under control condition (G0F0) having depression in saline condition compared to sweet corn grew in normal condition. It significantly increased after treated with GSH and GSH-rich nano fertilizer. G0F1 in saline condition presented shorter root length than in normal condition. This indicated root do not grow longer due to nutrients availability near to the root, so roots can absorb nutrients easily and at the same time influenced the grow of plant height. Other than that, the length of root G1F1 without NaCl was lower than with NaCl condition, a sign of root stress due to salinity.

Chlorophyll is a green pigment in plant, important for photosynthesis process. It helps plant to absorb energy from sunlight and convert water and carbon dioxide into oxygen and carbohydrate. Measurement of chlorophyll content is vital to access the photosynthetic capacity, developmental stage and productivity in plant. In figure c, G0 indicates without GSH while G1 with GSH and F0 without nano fertilizer while F1 with nano fertilizer. Chlorophyll content in sweet corn plant without salinity was significantly decreased after being applied by G1F0 and G0F1. Then, it was increased slightly with G1F1. In contrast, chlorophyll content in saline condition was fluctuated. It was higher in G1F0 compared to G0F0 (control). Other than that, G1F0 showed the highest chlorophyll content followed by G1F1, G0F0 and G0F1. This showed that, GSH works effectively in saline condition by reducing excess Reactive Oxygen Species (ROS) which is harmful to the plant cell. Organelles in plant cell like chlorophyll was maintain and stabilized. It can function effectively to produce oxygen and carbohydrate in photosynthesis process.

The effects of GSH rich nano fertilizer to reduce salinity stress on photosystem II (PS II) was examined by determine minimal fluorescence (Fo), maximal fluorescence (Fm) and ratio of variable fluorescence to maximal fluorescence (Fv/Fm). PS II is a protein complex consists of peripheral antenna system, order chlorophyll to absorb and use light energy to assist the transfer of electrons from water to plastoquinone during photosynthesis process.

Minimal fluorescence (Fo) is a minimum level of light absorbed by the plant to keep photosystem II (PS II) reaction open. According to figure d, Fo in plant grown without saline condition was rapidly rise after treated with G1F0 and G1F1 but decreased with G0F1 treatment. In comparison, Fo of sweet corn plant grown in saline condition roughly lower than Fo without saline condition. In saline condition, Fo in plant with G1F0 was slightly higher than G0F0. Besides, it was rapidly fall after treated with G0F1. This may related with chlorophyll content. Chlorophyll content of plant in saline condition treated with G0F1 was lowest due to improper development of chlorophyll pigment. Excess salt may disrupt the cell

membrane and growth of chlorophyll pigment in sweet corn plant. Hence, these cause Fo of plant grown in saline condition, treated with G0F1 lowest among other treatment. Finally, it was recovered by G1F1. Therefore, this result proved that GSH may works simultaneously with Nano fertilizer to improve extreme effects of salinity on plant through growth and physiology in order to increase the productivity.

Maximal fluorescence (Fm) is a maximum level of light absorbed by plant to close all PS II reaction centres'. Figure e roughly showed that Fm of sweet corn plant in both conditions was fluctuated. Based on the result, Fm of plant under saline condition treated with G1F1 was the highest followed by G1F0, G0F1 and G0F0. Fm treated with G1F0 was increased compared to G0F1 and G0F0 ( $p < 0.05$ ). G1F1 was significantly higher than G0F1. Therefore, this showed that GSH and Nano fertilizer increased the maximum level of light absorbed by the plant under salinity. GSH helps plant tolerate in salinity while Nano fertilizer increase the phyto availability of nutrients, thus support growth and development of plant. In normal condition (without NaCl), Fm of plant treated with G1F0 was the highest followed by G1F1, G0F1 and G0F0.

Fv/Fm is the ratio of variable fluorescence to maximal fluorescence. It indicates the efficiency of quantum and energy excited of photosystem II (PS II). In short, Fv/Fm under saline condition was found significantly lower compared to Fv/Fm in normal condition. Fv/Fm without any treatment showed depression under saline condition. Then, it slightly rises in G1F0 and fall in G0F1. G1F1 slowly recover Fv/Fm under salinity. So, it can be considered that, G1F1 play role to increase Fv/Fm of sweet corn plant under stress condition ( $p < 0.05$ ). The rhythm of Fv/Fm under normal condition was almost similar with saline condition.

Stomata are pores form by pairs of guard cells in plant tissue that allow for gas exchange (in and out of carbon dioxide and oxygen). Stomata aperture is the length of stomata open due to the changes of environment in order to limit the uptake of water and carbon dioxide assimilation. Refer to the figure g, stomatal aperture of plant grown in salinity showed significant difference while in normal conditions the aperture was insignificant differences.

Under stress condition, salts decrease stomatal aperture. However, G1F1 and G1F0 significantly increase the degree of stomatal aperture. Therefore, this may increase the flow of carbon dioxide into the plant cell as consequence; it increases the rate of photosynthesis. This may relate with the increment of maximal fluorescence and chlorophyll content in plant by the application of GSH-rich Nano fertilizer under stress condition ( $p < 0.05$ ). It can be concluded that, GSH may reduce harmful effects due to salinity to make sure plant cell and organ can function effectively, enhance growth and physiology of the plants.

Disorder caused by salinity on plants come from disruption of ionic equilibrium (influx of sodium ion ( $\text{Na}^+$ ) dissolves membrane potential and facilitates the uptake of

chloride (Cl<sup>-</sup>) down the chemical gradient. Furthermore, Na<sup>+</sup> is toxic to cell metabolism and has deleterious effect on the functioning of some of the enzymes. Moreover, high concentration of Na<sup>+</sup> causes osmotic imbalance, membrane disorganization, reduction in growth, inhibition of cell division and expansion. These also lead to reduce in photosynthesis process and production of ROS. The reduction was regulated by hormonal signals coming from the roots. Salt stress influenced water absorption by roots where it consequently disrupted the nutrient absorption and photosynthesis process. Moreover, internal injury caused by salt stress also effected the plants. (Munns, 2002; Mahajan & Tuteja, 2005).

Application of nano improved root length under salt stress (Siddiqui et al., 2014). This result was supported by Prasad et al. (2012), where peanut plant that treated with nano scale zinc oxide showed the highest plant height and longest stem. Suriyaprabha et al. (2012) stated in their previous research where the application of nano silica increased lateral roots and root length of maize. The size and shape of nano fertilizer gave significant and positive impact to the growth and physiology of the plants since their size is small and easy to be absorbed by the roots of the plant.

Chlorophyll synthesis in plants under salinity was improved when treated with nano-SiO<sub>2</sub> thus increased chlorophyll content (Siddiqui et al., 2014). Kalteh et al. (2014) explained in their previous experiment about the increase of chlorophyll content in Basils when silica nanoparticles applied. Jahan et al. (2014) emphasize that GSH enhanced the chlorophyll content and at the same time Fm and Fo of the leaves were increased. Fo and Fm are plant capacity to absorb sunlight. Plants grow under stress condition were getting sunlight but unable to use all energy. Light intensity of plant under stress condition were functioning optimum and sunlight sufficient but unable to convert into full energy. The application of GSH can support plant to convert more energy, as Fv/Fm increase, chlorophyll content also increase. Fv/Fm of *P. maritima* was decreased when exposed to the high concentration of salt. Oxidative stress in this species was prevented by activity of ascorbate-gluthatione cycle. High antioxidant activity enhanced salt tolerance of *P. maritima* (Hediye et al., 2007)

Oxidative stress caused by salinity inducing water deficit. As a result, stomatal close and decrease CO<sub>2</sub> availability and photosynthesis process (Sudhakar, 2001). Reduce in growth and physiological of the plants such as decrease in stomatal aperture due to H<sub>2</sub>O<sub>2</sub> accumulation are some of defence strategies for plants to conserve energy and adapt in salt stress (Chaparzadeh et al., 2004). Under salinity, plant unable to take sufficient water thus less stomata aperture compare to normal condition where the stomata fully open and can convert energy in photosynthesis. Jahan et al. (2008) and Chen et al. (2012) reported that treatment with GSH widened the stomatal aperture.

#### IV. CONCLUSION

In conclusion, nano fertilizer with GSH show better result on salinity condition where they may reduce salinity stress on growth of sweet corn plant. Moreover, GSH-rich nano fertilizer improved the growth and physiological parameters of sweet corn plant. Data for physiological and growth parameters show that there are significantly differences between the treatments at p<0.05. This research was an invention in agriculture to help farmers overcome crop yield loss due to the salinity and increase agricultural productivity in Malaysia.

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