IMPACTS OF NACL ON GROWTH AND NUTRIENTS CONTENT IN PINEAPPLE VARIETIES UNDER IN VITRO CULTURE

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ABSTRACT

The study was conducted with the aim to evaluate the effect of sodium chloride (NaCl) on growth and nutrients content of pineapple (Ananas comosus (L.) Merrill) varieties. Explants of pineapple plantlets were cultured on MS medium treated with either 0 (control), 34, 68, 103, 137, 171, 205 or 240 mM NaCl. Four weeks after culturing, the fresh weight (FW), dry weight (DW) and number of leaves (LN) were determined. The solution for determination of nutrients content were prepared according to dry ashing method and absorbances were detected by using ICP-OES to quantify the amount of nutrients present in leaves sample. The results show that the growth of pineapple under tissue culture condition was not inhibited by salt concentration below 103 mM NaCl. Salt concentrations at 205-240 mM reduced plantlets growth significantly, but did not completely inhibit it. The results of this study indicated that the increasing of NaCl concentration in culture medium lead to the reduction of ion potassium (K^+) in all pineapple varieties.

Keywords: *Salinity stress, pineapple, Ananas comosus, tissue culture.*

Introduction

The coastal areas of East Coast Peninsular Malaysia, namely Pahang, Terengganu and Kelantan, are largely covered with BRIS (Beach Ridges Interspersed with Swales) soil. The area is exposed to high level of salt deposit and faces with severe drought condition during the middle of the year. This kind of soil and climatic condition might contribute the salinity and drought stress in the plant that eventually inhibits the growth and results in low productivity to the crop grown in the area (Shamsuddin, 1990).

Salinity stress represents an increasing worldwide environmental problem for crop production. Approximately 6% of the world's total land area and 23% of the cultivated lands are characterized by various salt-degraded soils (FAO, 2005). Soil salinity reduces the ability of plants to adsorb essential nutrients (Waisel, 1977) and it is a major abiotic stress (Zhu, 2001) and constraint to crop production in the arid and semi arid areas of the world (Ashraff, 1994). Tanji (1990) reported that soil salinity can result from a number of different causes, both natural as well as resulting from human activity. Salts occur and built up in soils, and are present in all water, except rainwater. Salinity stress leads to a serious morphological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang, Luttge & Ratajczak, 2001). Plants respond to salt stress by restricting the uptake of salt and adjust their osmotic pressure by synthesis of the compatible solutes such as proline, glycinebetaine and (Greenway & Munns, 1980) and sequestering salt into the cell vacuoles for the maintenance of low cytosolic Na levels (Glenn, Brown & Blumwald, 1999; Blumwald and Grover, 2006).

Pineapple (*Ananas comosus* (L.) Merrill) is one of the most important fruit crop cultivated in the tropical and subtropical regions of the world. It is among the crops that show some level of resistance against the drought stress. Between the 1960s to 1970s, Johor was the leading state in Malaysia as a pineapple producer and this, ranked Malaysia as the world's third biggest canned pineapple producer, at a turnover exceeding RM 100 million annually.

Thus, to meet its domestic demand, Malaysia is planning to expand the pineapple plantation for the future by developing large-scale commercial pineapple growing areas under the region zone planning known as East Coast Economic Region (ECER). The project is developed and focused in states in the East Coast of Peninsular Malaysia with the main purpose of raising pineapple production and alleviating the imports of the fruit. With the development of the project, pineapple plantation land would increase from the present area of 15,000 ha to 30,000 ha or more in 2010 that could raise the country's pineapple production to one million tons a year (Xinhua, 2007).

Many approaches have been done to increase the productivity of the plant especially for adaptation in stressful environment. Nowadays, in vitro culture technique offers an alternative approach for developing tolerant crops, and nowadays it has become a rapid and precise means of achieving improved tolerance (Cushman & Bohnert, 2000). Moreover, tissue culture plant under investigation is reduced from the risk of attack by pest and disease. The selection of cultivars that are highly resistant to stress environment will play an important role in improving the crop growth and yield.

Currently, report on cultivation of pineapple in the BRIS soil is still lacking. Thus, the study was conducted through in vitro culture to determine the level of salt concentrations influence on growth and nutrients content in pineapple. The results of the study may provide useful information for better understanding of tolerance mechanism in the species under salinity stress. This will play a significant role in improving management practices in reducing salinity effect on the crop growing in saline area such as the BRIS soil area in the East Coast of Peninsular Malaysia (Chee & Peng, 1998). In addition, establishment of salinity stress tolerance of pineapple variety which is suitable for the BRIS soil cultivation is needed to support the industry. The new tolerance variety of pineapple cultivars which could withstand salinity stresses would play a significant role in overcoming the barrier in increasing the production of pineapples.

Materials and Methods

Preparation of Tissue Culture Stock

The sterilized buds excised from three varieties of pineapple plant known as Spanish (N36), Queen (Morris) and Smooth Cayenne (Sarawak) were cultured on MS (Murashige & Skoog, 1962) medium supplemented with 1mg/L BAP. The established plantlets were then micropropagated and kept as stock cultures for future study.

Culture Treatments and Growth Measurements

Standard MS medium supplemented with 1 mg/L BAP were prepared and poured in 25 mL test tubes. The MS medium were mixed with NaCl (used as a salinity agent) at either 0 (control), 34, 68, 103, 137, 171, 205 or 240 mM NaCl. The cultures were kept in growth room at 25°C under 14 h photoperiod with light source obtained from the inflorescent tubes. At four weeks after culturing, the cultured explants were taken out from the tubes, washed and blotted for counting the leaf number (LN) and measuring of fresh weight (FW). Then, they were dried in the oven at 60° C for 48 hours for determining the dry weight (DW). The solutions for determination of minerals content in leaves of pineapple varieties were prepared according to the dry ashing method (SIRIM, 1980). The presence of the nutrients in solutions was detected by using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), Varians Inc. The Multielement Atomic Spectroscopy Standard Solution 1 (Fluka, Switzerland) was used as a standard solution. Phosphorus content in leaves of pineapple varieties was determined using Yellow Vanado Molybdate methods (SIRIM, 1980).

Statistical Analysis

The experimental design was a complete block design with three replications. The recorded data were subjected to an analysis of variance (ANOVA), and the means compared among treatments were made by using Duncan New Multiple Range Test (DNMRT) at the $P \leq 0.05$.

Result

Effect of Salinity on Plant Growth

Figure 1 (refer to Appendix) indicated the growth patterns of pineapple varieties (N36, Morris and Sarawak) after four weeks culturing in MS medium supplemented with various NaCl concentrations. Upon four weeks of culturing, plantlets biomass (fresh weight (FW) and dry weight (DW) in all three varieties were found to be increased with increasing NaCl concentrations in the medium from 0 to 68 and 103 mM, but the biomass declined with further increase of NaCl concentrations. The FW of N36 shows a very significant increase (230%) from 0.373 g in the control to 1.232 g in 68 mM NaCl, meanwhile Morris and Sarawak FW had significantly increased (50.9% and 69.3%) from 0.611 and 1.053 g in the control to 0.922 and 1.783 g respectively in 103 mM NaCl. DW of Sarawak variety had significantly increased (141%) from 0.082 g in the control to 0.198 g at 103 mM NaCl and declined with further increasing of NaCl concentrations. At 240 mM the DW of Sarawak variety had greatly reduced to 0.024 g. The result also shows that DW of N36 and Morris significantly increased (120% and 61%) from 0.025 g and 0.034 g in the control to 0.055 g in 68 mM NaCl, but gradually decreased with further increasing NaCl concentrations, with 0.022 g and 0.024 g respectively in 240 mM NaCl. The DW was significantly higher in the Sarawak variety than in N36 and Morris varieties for all NaCl concentration, except in 240 mM NaCl where all variety shared the same amount of DW.

Plant height of pineapple was also affected by salinity. The plant heights in N36, Morris and Sarawak varieties were increased from 4.614, 2.100 and 2.867 cm in control treatment to 5.000, 3.620 and 3.700 (8.36%, 72.4% and 29.1%) respectively in 68 mM. The height, however, gradually decreased with further increase of NaCl concentrations. At extremely high salinity, 240 mM NaCl, the height of pineapple varieties decreased to 2.386, 1.900 and 2.067 cm respectively.

The presence of salt in culture medium also affected the number of leaves in the pineapple varieties. The mean values of the leaf number were significantly different among the varieties of N36, Morris and Sarawak for NaCl concentration, except in 205 and 240 NaCl concentrations. Generally, leaf number in N36, Morris and Sarawak are not much changed at low and moderate concentrations of NaCl (34- 137 mM), but significantly declined from 8.667, 13.667 and 18.000 in control to 1.667, 5.000 and 3.333 respectively in 240 mM NaCl. Among the three varieties, Sarawak variety had significantly higher number of leaf and increased to 20.33 in 68 mM NaCl.

Effect of Salinity on Nutrients Content

Figure 2 (refer to Appendix) indicated the nutrients content in pineapple varieties after treated with different salt concentrations. The increase of NaCl concentration in culture medium leads to the reduction of ion potassium (K^+) in all pineapple varieties. Although the amount of K in Morris increased at first from 63.290 mg/g DW in control to 75.620 mg/g DW in 34 mM NaCl, the amount decreased with further increase of NaCl concentration, similar to the other two varieties.

The amount of sodium (Na^+) in pineapple varieties were found to have increased with the increment of NaCl concentration, except the Na^+ content in Sarawak variety declined when 171-240 mM NaCl was used. In N36, Na^+ content increased from 17.209 mg/g DW in control to 91.127 mg/g DW in 240 mM NaCl. Na^+ in Morris increased from 23.5 mg/g DW in control to 83.268 mg/g DW in 240 mM NaCl. Meanwhile in Sarawak variety, Na^+ concentration increased from 20.464 mg/g DW in control to 82.205 mg/g DW in 137 mM NaCl and lowered to 45 mg/g DW in 240 mM NaCl. Among the three varieties, N36 had the lowest Na^+ content in 0-137 mM NaCl meanwhile Sarawak variety emerged as the lowest Na^+ content in high NaCl concentration of 171- 240 mM.

The distribution patterns of calcium (Ca^{2+}) concentration were similar to those of magnesium (Mg^{2+}) content for all varieties, where both ions mostly decreased with the increment of NaCl concentrations supplemented in the medium, albeit Ca slightly increased in 34, 68 and 103 mM NaCl for N36 (Fig.2C and 8D). In general, both ions are high in N36 and Sarawak varieties and low in Morris.

Phosphorus (P) content in N36, Morris and Sarawak were significantly reduced with increasing NaCl concentration in the treated medium. P content in N36, Morris and Sarawak reduced from 0.026, 0.030 and 0.033 mg/g DW in control to 0.019, 0.015 and 0.012 mg/g DW respectively in 240 mM NaCl. Among the varieties, Morris maintained the highest P content while Sarawak remained as the lowest P content particularly in medium with more than 103 mM NaCl (Fig.2E).

Salinity has also affected Na/K ratio in the pineapples varieties. The Na/K ratio in N36 and Morris greatly increased from 0.283 and 0.414 in the control to 5.472 and 4.255 in 240 mM NaCl. In Sarawak variety, the Na/K increased from 0.297 in the control to 2.013 in 137 mM NaCl. N36 variety showed a significant high ratio of Na/K as compared to other varieties.

Discussion

The results of the study revealed that the biomass, plantlet height and leaf number of pineapple varieties was reduced and deteriorated under extremely high salinity level (171- 240 mM NaCl). However, under low (34 and 68 mM) and moderate (103 and 137 mM) salt concentrations, the growth was not affected by the stress and even lead to an increase in biomass production and plant height in all varieties. These results are supported by the other reports mentioning that photosynthesis is not always reduced by salinity and is even stimulated by low salt concentration (Parida & Das, 2005). Increase in growth under salt concentrations were also reported by others for many plant species such as *Centaurium erythraea* Rafn. (a medicinal plant) (Silver et al., 2007) and *Cassio montena* (Patel & Pandey, 2007). In *Alhagi pseudoalhagi* (a leguminous plant), the leaf CO_2 assimilation rate increased at 50 mM NaCl and was not significantly affected by 100 mM NaCl (Kurban et al., 1999). Increase in biomass due to NaCl induced stress has been reported in maize (Sajid et al., 2007) which may be due to the increase in proline accumulations during salinity stress (Misra & Gupta, 2005).

Sodium content of the plantlets increased with increasing concentration of NaCl induced osmotic stress in the culture medium. This is similar to what has been observed earlier in a number of studies (Ahmad, Javed & Ashraf, 2007; Javad, 2002). In contrast, the K and Ca content decreased with increasing NaCl concentration. The same situation occurred in tomato where salinity increased Na and Cl and associated with decreasing in K and Ca contents (Maggio, Raimondi, Martino & Pascale, 2007). Plantlets that contained higher levels of Na are thought to regulate growth and help plant to tolerate high levels of salinity through ionic compartmentation (Ahmad et al., 2007; Lutts, Kinet & Bouharmont, 1996) and these regulations enable some plants to survive and growth better under salinity stress. The results from this study also found that Na/K ratio increased with increasing salinity concentration. The high levels of Na inhibit the K uptake and this leads to an increase in Na/K ratio (Cicek & Cakirlar, 2002).

According to Grattan and Grive (as cited in Pessarakli, 1993), the interaction between P and salinity is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinization in different species. Overlach, Diekmann and Raschke (1993) reported that, although P concentration is related to the rate of photosynthesis, but it decreased the conversion of fixed carbon into starch. Therefore, the reduction in P content lead to the decreasing shoots growth. Racette, Louis and Torrey (as cited Pandey, 2005) also reported that the decrease of P in root tissue strongly stimulates the formation of hairy roots and lateral roots in leguminous trees, tomato, spinach, rape and white lupin.

Conclusion

Recent studies show that tissue culture was used as one of the most effective methods of determining, selecting and improving of salt tolerant plants. The technique was applied with many plant species such as tomato, rice, alfalfa and sugarcane. Thus, the technique was applied in this study with the aim to determine stress tolerance of pineapple variety and for cultivation on sandy or BRIS soil which reported low fertility.

According to the results of the study, the Sarawak variety was considered as a saline tolerant variety for cultivation in salty conditions compared to N36 and Morris. Growth of Sarawak variety decreased under salinity stress but did not inhibit the plant growth. The same situation occurred in the other two varieties but the detrimental effect was more profound in Morris variety. The physiological effect was obvious in Morris where leaf growth was retarded, changed to yellow and wilted under moderate level of stress.

The results revealed that the pineapple varieties respond to salinity stress by showing changes in plant growth parameter (FW, DW and LN) and mineral elements, enabling it to perform well under salinity condition. The mechanism evolved in pineapple permits the plant to survive by reducing the growth rate when exposed to the high salinity stress (205 and 240 mM NaCl). The characteristics of the pineapple plant especially its leaf which is waxy and known as cuticle help the plant to survive and prevent water loss through evaporation from the leaf surface during stress condition.

Moreover, the water stress condition induced by low and moderate salinity and drought, which is expected to be similar with the condition with BRIS soil which is considered toxic to the other plants, proves to be beneficial to this species in increasing its productivity. The field test of selected salinity clone of pineapple on BRIS soil will help to verify their potential cultivation in the BRIS soil area which stretches along the East Coast of Peninsular Malaysia. In addition, the information obtained in this study should be very useful and meaningful to farmers in order to expand the functionality of BRIS soil as well as an alternative of acid sulphate (acidic) and alluvium soil that is commonly used for pineapple cultivation.

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APPENDIX

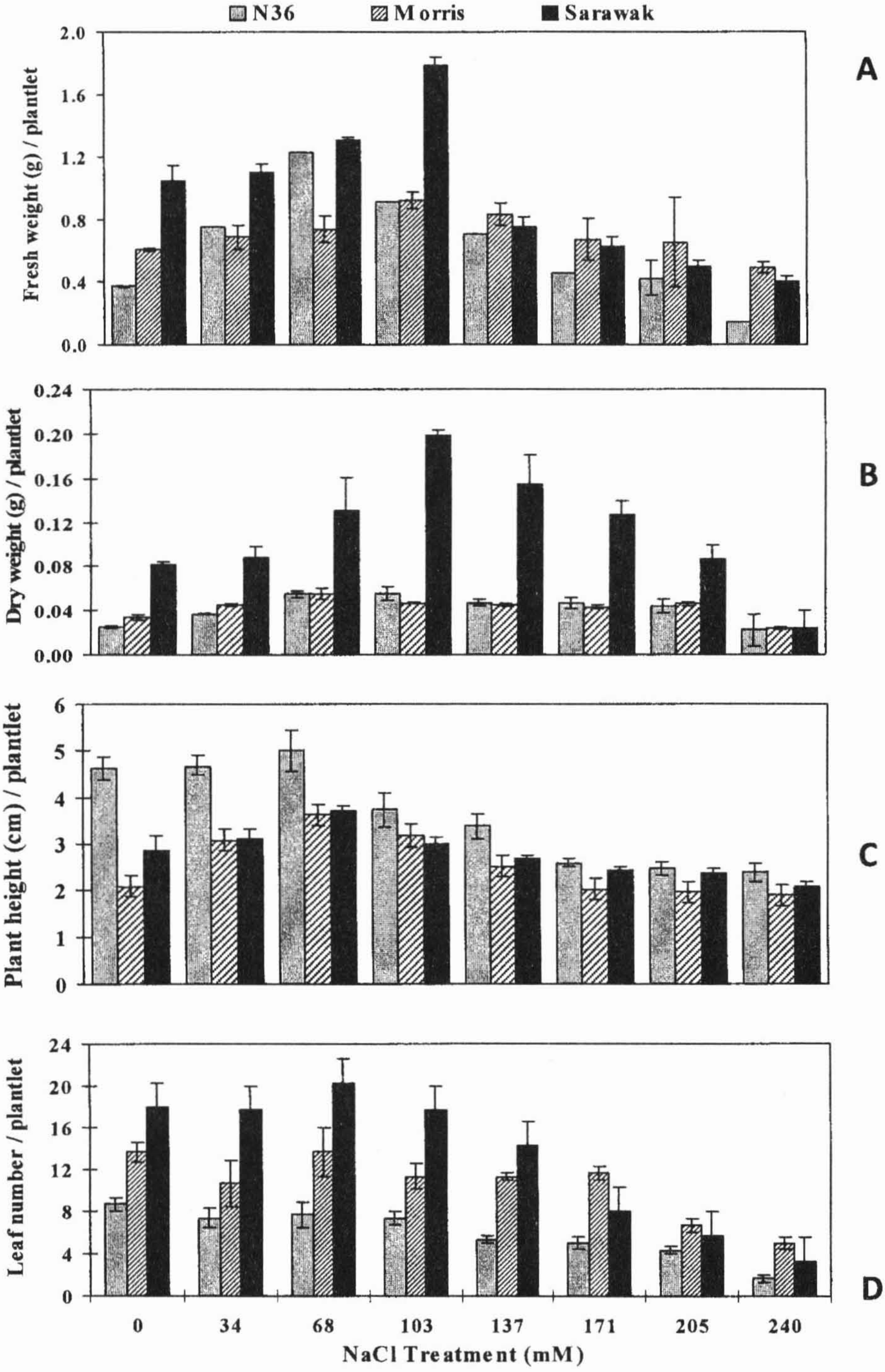

Figure 1: Effect of NaCl on (A) Fresh weight; (B) Dry weight; (C) Plant height and (D) Leaf number of pineapple varieties at four weeks after culturing. Each bar represents the mean (±S.E) and indicates statistically significant differences at $P \leq 0.05$ (DNMRT)

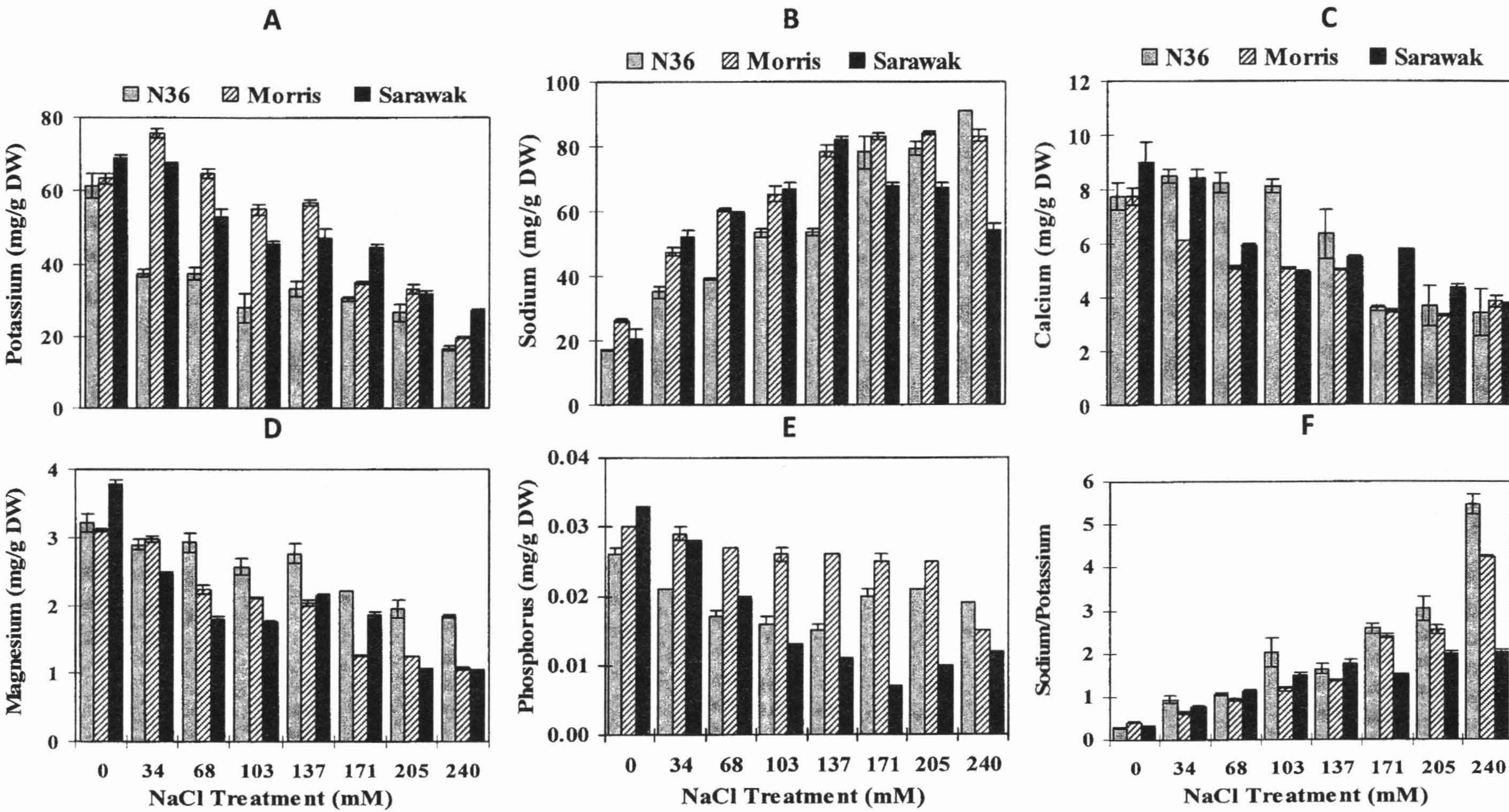

Figure 2: Effect of NaCl on mineral content of pineapple varieties at four weeks after culturing. Each bar represents the mean (±S.E) and indicate statistically significant differences at P≤0.05 (DNMRT)