DROUGHT STRESS TOLERANCE IN PINEAPPLE (*Ananas comosus.* L. Merrill) VARIETIES

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ABSTRACT

Pineapple (Ananas comosus (L.) Merrill) was recorded as a crop originated from the arid region of the Central America, and it is believed to be tolerant under drought condition and might be suitable to be cultivated in the BRIS (Beach Ridges Interspersed with Swales) soil area. The BRIS soil contained low water holding capacity, less nutrient and not suitable for cultivation of many crops species. Thus, a study was conducted to examine the effect of mannitol as a drought stress agent on three cultivated varieties (cultivar) of pineapple, namely N36, Morris and Sarawak, with the aim to determine and to select the best pineapple varieties for cultivating under the BRIS soil condition. The results indicated that the growth of pineapple was affected by different concentration of mannitol. The proline content of pineapple was increased with increasing of mannitol concentration however decreased at highest mannitol concentration. Sarawak and Morris varieties were suitable to be selected as the most suitable and profitable pineapple cultivar for cultivating in the BRIS soil area.

Keywords: *drought stress, pineapple varieties, proline, BRIS soil, mannitol*

INTRODUCTION

Drought is a prolonged period of deficient precipitation resulting in an extensive damage to crops and loss of yield (NDMC, 2008). In nature, water and soil are usually the most limiting factor for plant growth. If plant does not receive adequate rainfall or irrigation, the resulting drought stress can reduce growth of plants even greater than all other combined environmental stresses.

According to Mahajan and Tuteja (2005), drought stress caused the removal of water from the membrane and consequently disrupts the normal bilayer membrane structure and results in the membrane becoming exceptionally porous when desiccated. During drought stress, plants will response to water lacking by halting growth and reducing photosynthesis and change to other physiological processes in order to reduce water use (Gary, 2005). When water loss progresses, leaves of the plant may appear to change color, usually to blue green, foliage begins to wilt and fall and eventually died if the water stops resuming (Rodriguez, 2006).

The ability of the crops variety to perform better over other varieties under specific drought conditions or environment is known as drought tolerance (Anon, 2008). Plant response to drought condition showed many physiological and molecular processes such as changes in leaf senescence (Pic, *et. al.*, 2002), increase stomatal closure (Sahi, *et. al.*, 2006), altering gene expression (Dell' Aquila, 2004), inhibit photosynthesis (Flexas, *et. al.*, 2005), and increasing the accumulation of total soluble proteins and carbohydrates (Javad, 2002). These phenomenon could lead to the accumulation of free amino acids especially proline (Zhu, *et. al.*, 1998), enhancing the formation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals (Sunkar, *et. al.*, 2003), reducing water content and respiration rate (Galmes, *et. al.*, 2007) and consequently reducing the growth and yield of the plants. Proline is the most common osmolyte accumulated in plants tissue as a response to abiotic stress (Kaul, *et. al.*, 2006). Proline protects membrane, organelles, protein and other macro-molecule against severe damage due to high content of Na^+ and Cl^-. It also functions as a radical scavenger and permit influx of water into cytosol, and serve as an N reserve for utilizing during recovery (Sairam and Tyagi, 2004; Parida and Das, 2005).

Some species have anatomical or physiological characteristics that allow them to withstand drought or to acclimate to drought (Gilman and Robert, 1999). Pineapple is one of the crops that showing some level of resistant to a wide range of climatic and edaphic factors (Wang, *et. al.*, 2003). Although pineapples can be grown on most types of soils, the crop is however well adapted in the acidic soils condition (Sipes, 2000). The tolerant plants such as pineapples can defense against excessive water loss, because it has a special layer of water storage cells on the underside of the leaf that acts as a reserve in times of water stress. The epidermis of the pineapples leaves is thick and tough to resist wilting, prepared for the moment soil moistures become available (Anon, 2008b). According to Jia and Bartholomew (2005), drought stress had no effect on flower induction of pineapple, but significantly reduced pineapple fruit mass.

BRIS soil is well known for their inherently lower status compared to mineral soils (Lim, 2002). They have very low organic and moisture contents but high in soil temperature, leaching rate, hydraulic conductivity and evaporation of water from the soil surface (Sai, 2002). The pH of BRIS soil is low (<5) and thus acidic with water holding capacity is also low (4.5 cm/45 cm) (Khairuddin and Mohammad, 1992). The establishment of drought stress tolerance of pineapple variety which suitable for the BRIS soil cultivation is needed to support the pineapple industry. The new tolerance variety of pineapple which could reduce the effect of drought stresses in the plant would play a significant role in overcoming the barrier in increasing the production of the crops.

MATERIALS AND METHODS

Culture treatments and growth measurements

Pineapple planting materials were obtained from Department of Agriculture (DOA), Pontian Johor. Standard MS medium supplemented with 1 mg/L BAP were prepared and poured in 25 mL test tube. Drought treatment, the MS medium were mixed with mannitol, used as a drought agent at either 0 (control), 22, 44, 66, 88, 110, 132 or 154 mM mannitol. 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 oven at 60° C for 48 hours for determining the dry weight (DW). The methods described by Bates *et. al.* (1973) was used for proline extraction and quantification and the procedure was standardized using L-Proline (Sigma Aldrich, St. Louis, MO, USA) to quantify samples values.

Statistical analysis

The experimental design was a complete block design with three replications. The recorded data were subjected to 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 mannitol on plant growth

The effects of drought stress on fresh weight (FW), dry weight (DW), plant height and leaf number of pineapple varieties as imposed by mannitol treated in the medium were shown Figure 1. FW of N36 variety was increased from 1.240 g in control to 1.434 g in 44 mM mannitol, and gradually decreased to 0.44 g in 154 mM mannitol treated medium. FW of Sarawak variety was significantly reduced from 0.928 g in the control to 0.06 g in 22 mM mannitol, increased to 0.41 g in 66 mM and then remained stable with subsequent increase of mannitol concentration. Meanwhile, FW of Morris variety was reduced from 0.781 g in the control to 0.42 g in 22 mM mannitol but more or less remained stable with further increase of mannitol concentrations (Fig.1A).

Fig.1B shows the pattern distribution of DW in the N36, Morris and Sarawak pineapple varieties as effect by the eight different concentrations of mannitol. The result displayed that the DW was higher in N36 than Morris and Sarawak varieties under 22-110 mM mannitol. The DW of N36 significantly increased from 0.124 g in the control to 0.178 g in 22 mM mannitol and then declined to 0.3 g in 154 mM mannitol. In contrast, DW of Sarawak variety had significantly reduced from 0.075 g in the control to 0.02 g in 22 mM mannitol slightly increased to 0.05 g in 66 mM mannitol and then remained stable with further increasing of mannitol concentration. Differently, FW in Morris where more or less remain stable as in control between 0.3- 0.6 g in all mannitol concentration.

Fig.1C shows that plant height of N36 increased from 4.130 cm in control to 4.940 cm in 44 mM mannitol and slightly reduced with further increased of concentration. It also indicated that plant height of Morris and Sarawak increased from 2.633 and 2.867 cm in control to 3.733 and 4.833 cm respectively in 22 mM mannitol, and then decreased in fluctuation pattern in subsequent increase of mannitol concentration. Among the three varieties, Morris retained as the highest plant in all mannitol concentrations.

Fig.1D shows the effect of mannitol on leaf number of pineapple varieties. The result displayed that leaf number in N36 remained stable around the control, 9.20, in all mannitol concentrations. Meanwhile, leaf number of Sarawak variety decreased from 17.67 in control to the 9.67 in 22 mM mannitol and increased to 19.33 in 66 mM mannitol, and decreased again with further increased of mannitol concentration. Morris variety contained the lowest leaf number which was significantly reduced from 12.5 in the control to 3.5 in 22 mM mannitol and continuously remained stable with further increase of mannitol concentration.w

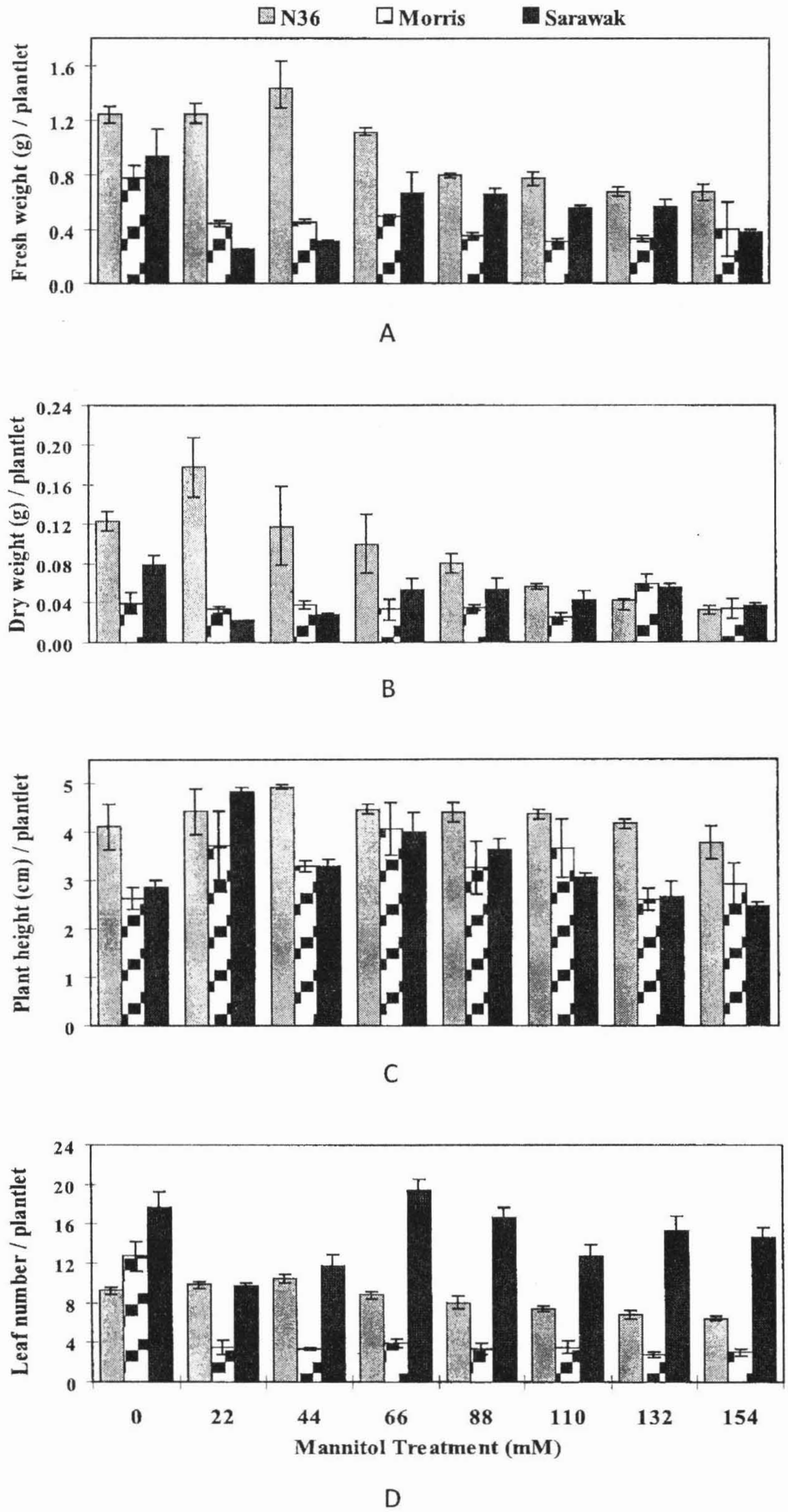

Figure 1: Effect of mannitol on (A) Fresh weight; (B) Dry weight; (C) Plant height and (D) Leaf number of pineapple varieties after four weeks. Each bar represents the mean (±S.E) and indicate statistically significant differences at $P \leq 0.05$ (DNMRT).

Effect of mannitol on proline content

Fig.2 displays the pattern of proline content in the pineapple varieties as affected by different concentration of mannitol used as a drought agent. Generally, proline content increased with the increasing mannitol concentrations for up to 110 mM, but reduced in 132 and 154 mM mannitol. At 110 mM mannitol, proline content of N36, Morris and Sarawak varieties increased from 4.174, 4.647 and 3.56 mg/g FW in control to 4.85, 5.295, and 5.613 mg/g FW respectively. Among the varieties, Morris always remained as the variety contained the highest proline content in all mannitol concentrations.

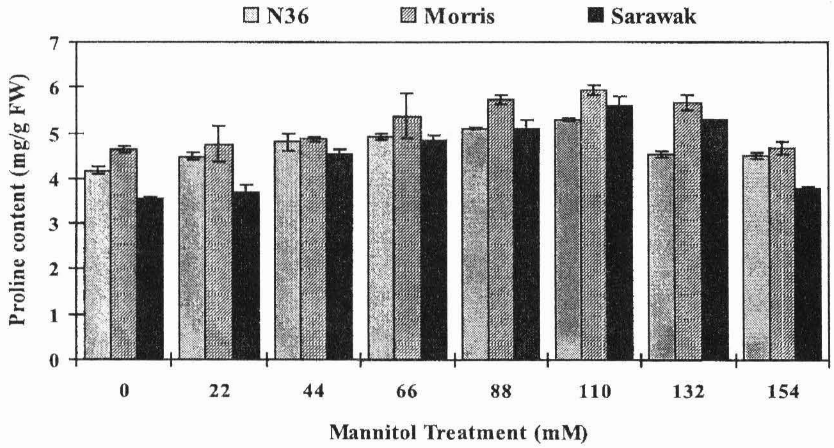

Figure 2: Effect of mannitol on proline content of pineapple varieties after four weeks. Each bar represents the mean (±S.E) and indicate statistically significant differences at P≤0.05 (DNMRT).

DISCUSSION

The present study was designed to determine the effect of mannitol concentrations as a water stress agent to the growth of pineapple (*Ananas comosus*). The result showed that growth of pineapple varieties was found to be affected more favorably by an increasing supply of mannitol concentrations. Most of the growth parameter such as plant height, leaf number and biomass (fresh weight and dry weight) decreased by increasing the mannitol concentrations in the culture medium.

Among these three varieties, N36 exhibits better performance in high mannitol concentration. The plant height, leaf number and fresh weight of the plant were highest at 44 mM mannitol compared to Morris and Sarawak. The plant height increased about 110% of control followed by leaf number 113% and fresh weight 116%. However, dry weight of the plant showed maximums at mannitol concentration 22 mM and it increased about 144% of control.

In contrast, mannitol concentrations have no effect on growth of Morris. The result obtained shows that no significant differences were found among all concentrations to plant height, leaf number and fresh weight of Morris. On the other hand, the dry weight of Morris was significant at mannitol concentration 132 mM and it increased about 148% of control.

In Sarawak variety, mannitol concentrations have no effect on biomass, but the concentration at 22 and 66 mM increased the plant height and leaf number of the variety by about 169% and 109% of control respectively. The result seem to be consistent with other research which indicated that water stress reduced plant biomass such as in *Catharenthus roseus* variety alba and rosea (Jaleel, *et. al.*, 2008). There were several possible explanations for these results. The growth of plant was influenced by many factors included internal and external factors. Plant will react to changes in environment to cope with stress conditions. The reduction in biomass may be due to the decrease of plant growth and photosynthesis during drought stress.

The reduction in plant height might be associated with decreasing in cell enlargement and cell growth due to the low water turgor pressure and also more leaf senescence under drought stress. The event also may cause decreasing in leaf number and as well as reduced leaf sizes, leaf longevity includes leaf area and expansion. The same event has been discovered in other species such as potato (Deblonde and Ledent, 2001). The results were following the statement of Jaleel *et. al.* (2008) which found that water stress affected quality and quantity of plant which grew depending on cell division, enlargement and differentiation, and plant response to drought stress by showing reduction in water content, turgor, total water potential, wilting, stomata closure and decreased in cell growth .

The results also indicated that drought stress encouraged proline accumulation in pineapple varieties. Proline concentration was low in the leaves of control plant compared to treatments during plant development. The same events also occurred and reported in many plants such as sunflower (Unyayar, *et. al.*, 2004) and rice (Hien, *et. al.*, 2003). Obviously, mannitol concentrations increased proline contents about 108% to 127% of control in N36, 102% to 128% in

Morris and 103% to 158% in Sarawak. The increased in proline content was obtained in mannitol concentration from 22 mM to 110 mM. However, proline contents in each variety decreased to 109% and 108%, 122% to 101% and 149% to 107% respectively at high mannitol concentration of 132 and 154 mM.

Large numbers of compounds such as proline, fructans and glutamate, glycine-betaine, carnitine, mannitol, sorbitol, were synthesized during stress, which play a key role in maintaining the osmotic equilibrium and in the protection of membranes as well as macromolecules (Mahajan and Tuteja, 2005). Proline was one of the amino acids, which appear most commonly in responsed to stress. Proline within the cell can act as an osmolyte with high compatibility for enzymes and other cell macromolecules, therefore protecting them from drought-stress induced damage. Osmotic adjustment produced by proline caused a drop of the osmotic potential in plant tissues (Hare, *et. al.,* 1998). Plant accumulated proline during drought stress in order to associate positively with recovery resistance by serving as a source of energy to the recovering plant. In order to deal with such effect, plants also have evolved a number of protective scavenging or antioxidant defense mechanisms (De-Ronde, *et. al.,* 1999). Many authors also suggested that accumulation of proline during drought stress might be contributed to the detoxification of the active oxygen species. Some believed that proline involved in the maintenance of membrane integrity as an adaptation to water stress conditions which supported the roles of proline as osmoregulator. Accumulation of proline as a response to drought stress was found in many plant species such as wheat (Hamada, 2000), sunflower (Manivannan, *et. al.*, 2007), alfalfa (Akhondi, *et. al.*, 2006), rice (Ahmad, *et. al.*, 2007) and sugarcane (Errabii, *et. al.*, 2006).

CONCLUSION

In conclusion, variety Sarawak was considered as drought tolerance variety for cultivated on water stress condition compared to N36 and Morris. Growth of variety Sarawak was reduced under drought stress but did not inhibit the plant growth. The same situation occurred in other two varieties but the detrimental affected was more profound in Morris variety. The physiological effect was obvious in Morris which leaves started to retarded and change to yellow colour and wilting under moderate level of stress. Finally, the growth was reduced and plant died after prolong exposure to drought stress condition.

The results revealed that the pineapple varieties response to drought stress by showing changes in plant biomass, plant height, leaf number, content of proline, malate and mineral elements as well as chlorophyll pigment by enhancing CAM photosynthesis that enabling it to perform well under drought condition. The mechanism evolved in pineapple permits the plant sustains the survival by reducing the growth rate when exposed to the high drought (132 and 154 mM mannitol). The characteristics of the pineapples plant especially its leaf which is waxy and known as cuticle help plants to survive and prevent water loss evaporation from the leaf surface during stress condition.

Moreover, the water stress condition induced by low and moderate drought, which is expected to be similar with the condition under the sandy or BRIS soil near the sea which consider toxic to the other plants, provides a beneficial to this species to increase its productivity. The field test of selected salinity and drought clone of pineapple on BRIS soil will help to verify their potential cultivation in the BRIS soil area which is stretch along the East Coast of the Peninsular Malaysia. In addition, the information obtained in this study supposed to be very useful and meaningful to farmer in order to expand the functional of BRIS soil as well as an alternative of acid sulphate (acidic) and alluvium soil that commonly used for pineapple cultivation. The knowledge generated through these studies should be utilized in making transgenic plants that would be able to tolerate stress condition without showing any growth and yield penalty.

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