Will Pineapple (*Ananas comosus L. Merr.)* be a successful crop with global warming- induced temperature and water stress in Sri Lanka?

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Abstract - *Global warming is taking place rapidly mainly due to anthropogenic and natural activities and will have adverse effects on plant growth and development of crop plants depending on the atmospheric temperature and soil moisture level. When the temperature exceeds the optimum and also when there is a water stress, plants respond negatively showing a sharp decline in growth and development which would ultimately affect the yield. In vitro propagation is a widely used vegetative propagation technique to obtain clones of true-to-type daughter plants. This mass scale propagation is used around the world and also in Sri Lanka using different explants, since it produces daughter plants with beneficial characteristics such as high yield and early fruiting.This study intends to investigate the impact of simulated temperature stress and water stress reflecting global warming on in vitro propagated pineapple plants. It is presumed that this research would help in planning in the cultivation of pineapple to obtain a substantial yield. This study was conducted in two locations in the Open University of Sri Lanka premises viz. in a polytunnel at an elevated temperature of 35 °C and in a plant house under the ambient temperature. In vitro propagated pineapple plants obtained through ratoon suckers were used for this study. In each location there were two sets of plants maintained under two water regimes. One set was maintained under 100% soil moisture content while the other set was maintained at 50% soil moisture content. The plants were watered daily to their respective water capacities and maintained in completely randomized design. All the experiments were repeated twice to justify, the replication of temperature effect by the poly tunnel. The mean values of the vegetative, yield and quality parameters were taken for statistical analysis.According to the results, shoot initiation and proliferation of pineapple was successful on MS medium fortified with 2.5 and 4.0 mg/l BA respectively using ratoon suckers. Rooting of the proliferated shoots was carried out using MS medium with 1.0 mg/l IAA.The vegetative growth of pineapple was successful even under stressful conditions. The fruit diameter was high under both temperature stress and water stress condition. The fruit weight was also satisfactory under both temperature stress and water stress. The firmness of flesh was low under stressful conditions, which could be considered as a favorable characteristic from the consumers' point of view. The pH values of fruits on exposure to stress were less acidic compared to the pH of the fruits under no stress condition which would be a positive character from the consumers' point of view. It will also help to have good post-harvest quality due to less microbial activity. The total soluble solids were low on exposure to stress but it was not significantly different from other treatments. Fruits with large diameter, soft flesh and which are less acidic under both temperature stress and water stress prove that even under the simulated global warming conditions pineapple yield and quality would not be affected. Therefore C_4/CAM plants have more positive effects towards global warming due to their physiological advantage.*

Key words: *Pineapple, Temperature stress, Water Stress, Yield*

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1 INTRODUCTION

The temperature increment due to global warming is a major problem faced by the world today. According to the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC, 2014), the cause of this is mainly the anthropogenic emission of various greenhouse gasses such as carbon dioxide, methane and nitrous oxide, which are emitted at an increasing rate with the economic and population growth of the world since the pre-industrial era.

Sri Lanka would also face consequences of this temperature increment. As reported by Chandrapala and Fernando (1995), there was an increase in temperature in Colombo by 0.0164 ^{o}C year during the period of 1961 to 1990. It was predicted by General Circulation Model (HadCM3) that the annual average global temperature would increase. During May to September, the south west monsoonal period, the annual air temperature across Sri Lanka is predicted to be increased by 1.6 ^{o}C (A_2 scenario) and by 1.2 ^{o}C (B_2 scenario). Further, it is predicted that north east monsoon rainfall also would decrease in the coming years (De Silva *et al.*, 2007).

Sri Lanka isexperiencing these effects even now, because temperature related extreme indices have risen over most parts of the country and annual average rain fall has been decreasing at a rate of about 7 mm per year, for the last 57 years (Ranasinghe *et al.*, 2014).The increase in temperature and the decrease in rainfall would affect growth and development of plants. Plants would be subjected to temperature as well as water stress due to increase in evapotranspiration. The agricultural activities which involve plant growth and development would heavily be affected by climate.

Chapin *et al.*, (1987) reported that temperature and soil moisture are important factors which determine the growth and productivity of plants. De Silva (2006) has predicted that increase in temperature will lead to increase in soil moisture deficit and additional irrigation water would need to satisfy the evapotranspiration. Dishani and De Silva (2013) also reported that the rate of plant growth and development depends on the surrounding temperature of the plant and each plant species has a specific temperature range represented by minimum, maximum and optimum temperatures. When the temperature exceeds the optimum level required for biological processes, the plants would respond negatively, especially showing a steep decline in the growth and yield (Cynthia and Hillel, 1995). According to Pastori and Foyer (2002) an increase in one degree above normal would lead to a significant reduction in growth and yield of a plant.

In this study, pineapple was selected because pineapple is the second most important fruit crop worldwide, after banana, contributing to over 20% of the production of tropical fruits (Coveca 2002). Pineapple (*Ananascomosus*L. Merr.), belongs to the family Bromiliaceae. is a plant found mainly in tropical and subtropical regions of the world. The fruit is a composite fruit and the ripened fruit of this plant is very popular among people as a dessert and syrups extracted from the fruit can be used as a juice or in wine production. In animal food, leaves can be used in three forms: fresh, dried and in silage (Coppens and Leal 2003).The fruit residue after extracting syrup is used as a cattle feed or fertilizer. The use of byproducts of pineapple culture in feed production, canning and juice extraction has been encouraged. Pineapple may offer additional advantages, such as its relevance as fiber source. Leaves are important in obtaining fiber which are used to weave cloth and in paper industry.Pineapple fiber has numerous qualities, such as its texture, its length (up to 60cm), high water and dye holding capacity, high whiteness, brightness, resistance to salt and tension strength (Argan *et al*, 2009).

In addition, *Ananas* sp. has medicinal properties as well. The ripe fruit has germicidal, laxative and invigorative properties since it contains the enzyme bromelain (Tochi *et al.* 2008). Bromelain has been produced for use as a meat tenderizer and as a component of pharmaceuticals. Due to the presence of this enzyme, pineapple is an appetizer. This fruit is also used in treating cardiovascular disorders. Unripe fruit can cause abortions. Further, pineapple is exported to other countries and Sri Lanka earns foreign exchange.There are two commercial varieties in Sri Lanka, i.e., Kew and Mauritius, out of which Mauritius is preferred by farmers and consumers. However, Kew variety has a better export market as a fruit which is used for obtaining syrup and in the jam industry.

1.1 Impact of Climate Change on Tissue Cultured Plants

A major problem that both large scale commercial production of pineapple and the expansion of the existing small farms face is the difficulty in obtaining uniform planting material in large quantity due to the low rate of multiplication by conventional methods and the lack of high quality propagules. Pineapple propagation is performed asexually, using shoots from different parts of the plant, such as bulbs, crowns and axillary buds. Recent advances in plant biotechnology methods applied to pineapple crops enhanced their potential application, both for basic studies and for direct application in agriculture (Read, 2007; Aragón *et al*. 2009; 2010).

In vitro propagated plants are usually produced by obtaining explants from selected healthy mother plants, having desirable characteristics such as ability to withstand stress, producing high yield and disease resistance. Further, they would inherit these beneficial characteristics from their parent plants, and would be expected to be affected to a lesser extent by the stress. Furthermore, with proper manipulation, a clone of thousands of true- to- type plants could be obtained through micropropagation. With the selection of disease-free mother plants and proper selection of explants, daughter plants which are also disease-free would be resulted.

In addition, seasonality would have no or minimum effect on the growth of the plants, when grown under growth room conditions. However, once the plants are acclimatized and transferred to the field, seasonality would have an impact on the growth and development of the plants. Cost of production is the only drawback of *in vitro* propagation. However, this could be minimized by producing a clone with a large number of plants and adopting a low cost application of this technique to reduce the cost of a micropropagule, without compromising the quality.

1.2 CAM Photosynthesis in Pineapple

Crassulacean Acid Metabolism (CAM) is a photosynthetic pathway identified in pineapple plants. Although the designation of CAM derives from the *Crassulaceae* family, the *Ananascomosus*is belonging to family *Bromeliaceae is* the CAM species with the highest commercial value. CAM photosynthesis appears to have originated as a means to scavenge respiratory CO_2 under conditions where the carbon balance is restricted, in environments where water availability becomes temporarily or seasonally constrained, such as deserts or rock outcrops. CAM metabolism causes major changes to leaf structure, succulence being the most obvious innovation as it facilitates the capture of night-time CO_2 released by respiration (Guralnick *et al*. 2001). Plants with CAM metabolism have a complex balance of C_3, C_4 and CAM photosynthetic pathways are well characterized but there are still a few metabolic details not well understood (Willert *et al.* 2005). The interpretation of the C_3/CAM transition as a complex interaction between environment and metabolism, in opposition to a basic molecular interpretation of the circadian cycle linked to carbon metabolism are still elements to be clarified on pineapple plants.

This research carried out to study the effect of global warming on *in vitro* propagated plants is very important, since no research of this kind has been carried out in Sri Lanka or worldwide up to now. Furthermore, a research on impact of global warming on the plants selected pineapple has not been conducted so far. Therefore, this research would be vital to those who are involved in the field of tissue culture as well as cultivation of pineapple. To date, there are no published data which would allow the prediction of growth and yield reductions of pineapple crop as a result of varying levels of water stress and temperature stress. So far, most studies of plant cold stress at the molecular level have focused on C_3 and C_4 plants, while research on CAM plants has remained scarce. This study will aid in understanding the temperature stress response in pineapple and other CAM plants.

This study focuses on the adaptive measures developed by plants for increased temperature and decreased rainfall or increase in soil moisture deficit due to global warming. Therefore, temperature stress is induced in temperature regulated polytunnel and water stress is induced by the amount of water applied (i.e: field capacity level and 50% of the field capacity level of soil moisture) and the effect of temperature and water stress on growth and yield parameters were measured. The main aim of the research project was to study the impact of induced temperature and water stress on vegetative and reproductive characteristics of the plants, developed through tissue culture technology, using Pineapple *Ananas comosus as* the model plant.

2 METHODOLOGY

2.1 *In vitro* propagation

The glassware required for preparation of media, sterilization and inoculation of the explants were washed with the commercial liquid detergent and running tap water and allowed to dry in a dryer. The glassware was then sterilized in a hot air oven at 180 °C for two hours. The metal equipment such as scalpels and pair of forceps was washed with the commercial liquid detergent and running tap water. These were then wrapped in aluminium foil and sterilized in a hot air oven at 180 °C for two hours. After each use during inoculation, the metal tools were dipped in 70% ethyl alcohol and flamed. The bench and the inner walls of the laminar flow cabinet were cleaned with 70% ethyl alcohol before and after use (Nagahawatte *et al.*, 2014).

Modified Murashige and Skoog's medium, (MS medium; Murashige and Skoog, 1962) was used as the basal medium. Different growth regulator combinations were incorporated into this medium depending on the requirement. In addition, Bacteriological agar and sucrose were also incorporated into each medium as a gelling agent and a carbon source respectively.

Different types of media necessary for shoot initiation, proliferation, sub culturing and rooting were prepared accordingly and the pH of the media was adjusted to 5.8±0.1 with the use of 0.1 N NaOH or 0.1 N HCl. 25 ml of the medium was dispensed into sterilized jam bottles, covered with previously autoclaved polypropylene and secured with rubber bands. The bottles containing media were autoclaved at 121ºC temperature and 1.05 kg/cm^2 for 15min. One month old ratoon suckers from mother plants were selected as the explants. The mother plants were brought from CIC Agri Farm Pelwehera, Dambulla and grown in the home garden(Nagahawatte *et al*., 2014).

The suckers were washed thoroughly with water to remove dust particles, and some leaves in the outer whorls of the plants were removed. The height of structures was reduced by cutting the stem and the leaves, leaving only two to three leaves. These were then washed thoroughly in running tap water by immersing in a beaker containing water and a few drops of the detergent Teepol. They were surface sterilized by immersing in a 50% commercial bleach solution (5.2% Sodium Hypochlorite) for 10 minutes, shaking occasionally, under the laminar flow hood. This was followed by rinsing three times in sterilized distilled water (Nagahawatte *et al.,* 2014).

The inoculum was made into a structure of 1.5 cm x 1.5 cm x 2.5 cm under the laminar flow cabinet and inoculated into a MS basal medium incorporated with 2.5 mg/l BA for shoot initiation of pineapple (Figure 1). The cultures were incubated under 16-h photoperiod at 25ºC with light intensity of 3000-4000 lux, in a culture room (Nagahawatte *et al*., 2014).

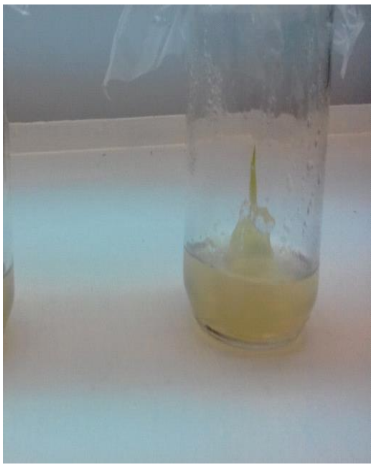

Fig.1.Inoculation of the Explant of Pineapple (Shoot Meristem of a Ratoon Sucker)

Initiated shoots were separated into individual shoots and inoculated into MS basal medium with 4.0 mg/l BA and incubated under conditions similar to initiation (Nagahawatte *et al*., 2014).Shoots were transferred to MS basal medium incorporated with 1.0 mg/l IAA and maintained for five weeks to obtain roots. Char coal was added to the medium to enhance rooting.

The plantlets were washed with tap water to remove traces of agar, separated into individual plantlets and potted in pots containing soil. These plants were maintained in a plant house at ambient temperature and watered daily for acclimatization. The acclimatized, *in vitro* propagated pineapple plants were potted, one plant each in pots having a diameter of 0.5 m, in a potting medium of top soil, coir dust and compost in 1:1:1 ratio. All the pots were filled with this mixture in equal amounts.

2.2 Temperature Regulated Poly Tunnel as Experimental Unit

The poly tunnel was constructed in the Open University premises, Nawala, in which the maximum daily temperature was maintained at 35 ºC. The poly tunnel was constructed in the direction of North-South to prevent the effect of mutual shading. The floor area of the tunnel was approximately 6.7 x 3.3 m^2 and the top was semi-circular elongated in shape (Figure 2). The basic structure was constructed with galvanized iron (GI) pipes and covered with UV treated polythene having the gauge of 120 microns. There was a manually operated door to access tunnel. The top of the tunnel has a semi-circular roof which has an opening enabling air circulation to maintain near natural conditions of relative humidity and CO_2 concentration. The above conditions satisfied simulation of high temperature inside the tunnel. However, in order to prevent the temperature from rising above the set temperature of 35 ^oC, there were two exhaust fans and a thermostat installed. When the internal temperature increased above the set temperature, the automation of fans would bring the temperature down to 35 ^oC.

(a) (b)

Fig. 2. (a)The External and (b) Internal View of the Polytunnel in which the Plants were maintained at a Maximum Temperature of 35 ^oC

The variation of temperature inside the polytunnel and the ambient temperature outside over a period of 24 hours was measured (Fig. 3). The temperature at night falls below the maximum temperature set for that particular poly tunnel to represent the diurnal variation. However, the temperature maintained inside the poly tunnel was always higher than the ambient temperature; therefore temperature stress was enforced on the plants during day time while there was photosynthetic activity.

2.3 Experimental Conditions

All the pots were maintained in a poly tunnel at a maximum daily temperature of 35 ºC using a thermostat and two exhaust fans. The pots containing pineapple plants were individually divided into two equal groups. Two water regimes were imposed on the two sets of plants. They were, watered to 100% field capacity level and 50% of the field capacity level, imposing a water stress. Soil moisture measurements were made using tensio- meters planted in the pots regularly. The plants were maintained in a completely randomized design. The plants of pineapple were manured and maintained except for water management, according to the recommendation of the CIC Agri Farm.

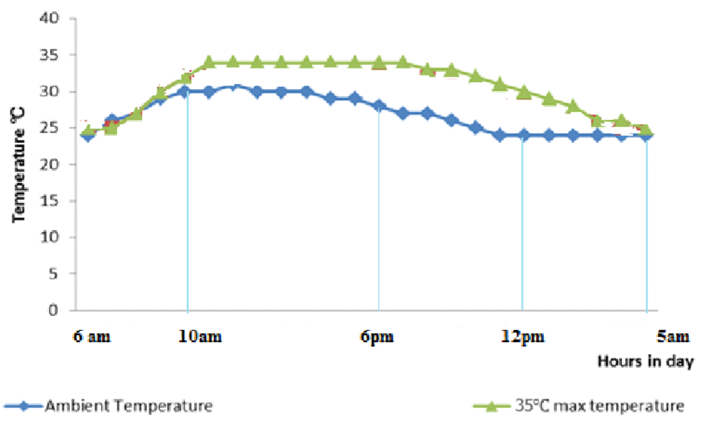

Fig. 3. Temperature Variation Inside and Outside the Polytunnel

The four treatments are as follows:
T_1- temperature stress, no water stress (in the poly tunnel at maximum temperature of 35 °C and watered to 100% soil moisture level)
T_2- temperature stress, water stress (in the poly tunnel at maximum temperature of 35 °C and watered to 50% soil moisture level)
T_3-no temperature stress, no water stress (in the plant house at ambient temperature and watered to 100% soil moisture level- control)
T_4 – no temperature stress, water stress (in the plant house at ambient temperature and watered to 50% soil moisture level).

2.4 Measurement of Parameters

2.4.1 Growth parameters

Immediately after the plants were transferred to each location, and at four week time intervals, vegetative growth parameters such as mean height of plants, mean number of functional leaves, mean length of leaves of pineapple were measured. These measurements were recorded in all four treatments of pineapple plants.

2.4.2Yield Parameters of Pineapple

The time taken for flower initiation and the time taken for fruit ripening were recorded for each pineapple plant. When the fruits have ripened, they were picked and the weights with and without the crowns were recorded using a digital balance. The lengths with and without the crowns were measured using a meter ruler. The diameter of fruits at the widest point of each fruit was recorded using a Vernier caliper.

2.4.3 Quality Parameters of Pineapple

- Fruit Firmness

The firmness of the flesh of the fruits was measured using a Penetrometer. The mean value of three readings was recorded as the fruit firmness measurement.

- pH of the Fruit Juice

The quality parameters of pineapple fruit were evaluated by extracting the juice of the fruit using a mechanical blender. The pH of the juice was assessed using a bench pH meter- (Hanna pH 211 Micro Processor). The mean value of three measurements was expressed as the pH value of each fruit.

- Total Soluble Solids

The total soluble solids of the fruit juice extracted were measured using a digital UV-refractometer. The mean value of three readings was expressed as the Brix value. When the measurements were taken, equal number of drops of the extracted juice was placed on the refractometer prism plate and the readings were noted. After each reading, the prism plate was rinsed with distilled water and wiped with a soft tissue.

2.5Experimental Design and Data Analysis

All the experiments were carried out on the experimental design of completely randomized design (CRD). Due to the death of the plants during acclimatization, the number of replicates in pineapple had to be limited to three. ANOVA and hypothesis testing were carried out to compare the means of the parameters related to growth using SAS University software (University version). All the analyses were carried out at least in duplicate and in randomized order with the mean values for each treatment used three replicates to reduce random error.

3 RESULTS AND DISCUSSION

3.1 *In vitro* Propagation of Pineapple

One month after inoculation, the shoot initiation took place on the explant of pineapple (Figure 4).When the initiated shoots were transferred into a MS basal medium with 4.0 mg/l BA, in approximately 1 ½ months, 6-8 shoots/explant were developed from the sub-cultured shoots.

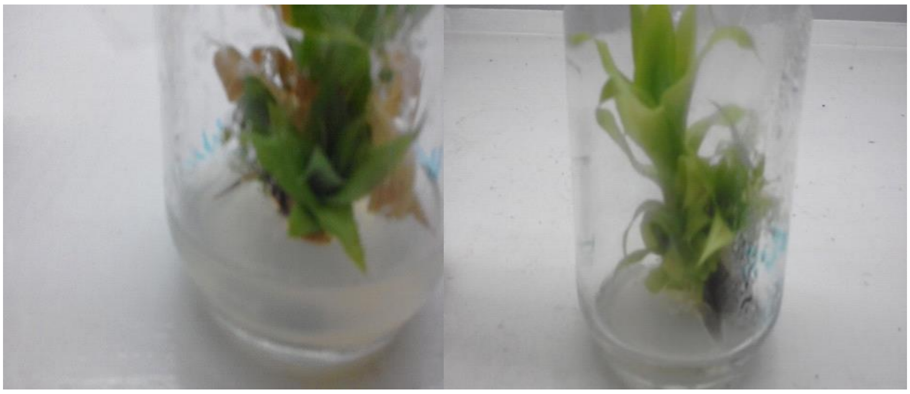

Fig.4. (a) Shoot Initiation (b) Shoot Proliferation

When the proliferated shoots were separated and transferred to the rooting medium viz,. MS basal medium incorporated with 1.0 mg/l IAA and charcoal, rooting of shoots took place in approximately 1 ½ months (Figure 5).

The process of acclimatization was successful. The process of acclimatization took approximately 1 ½ months. The plants were transferred to the poly tunnel and the plant house, after the acclimatization process, for the treatments to be imposed.

Fig. 5. (a)Rooting of Pineapple Shoots (b).Pineapple plants after Acclimatization

3.2 Growth Parameters

3.2.1 Height

The plant height has shown an increasing trend pattern from week 0 to week 80. The highest plant height was shown in plants under the treatment of temperature stress and water stress. The plants exhibiting the lowest plant height varied over the weeks.

According to Figure 6 which shows the plant height after 80 weeks, highest plant height was shown in plants which were under temperature stress and water stress. This was significantly higher than when compared with the other treatments. The lowest plant height was shown in plants of the treatment with no temperature stress but with water stress. However, there was no significant difference among heights of the plants of the other three treatments viz., temperature stress with no water stress, no temperature and no water stress and no temperature stress but with water stress. Further, there was no interaction effect of both the stresses on the height of plant according to the ANOVA.

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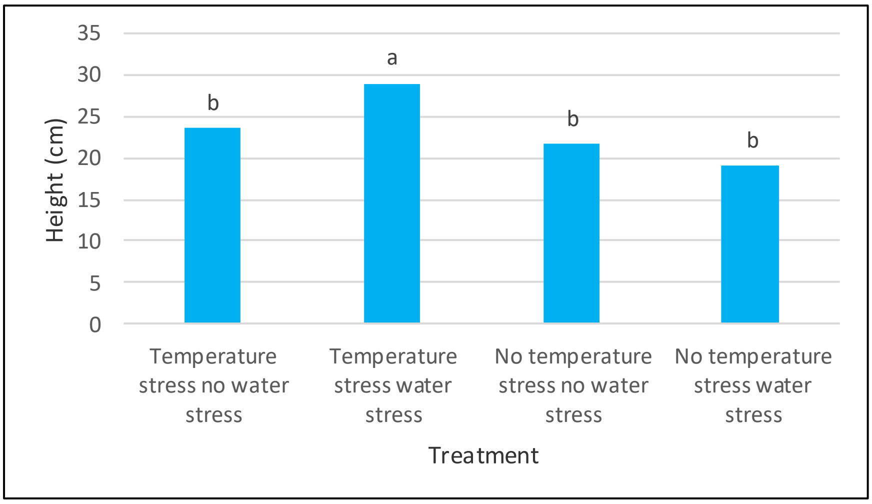

Fig. 6. Plant Height, after 80 Weeks

Pineapple being a plant with crassulacean acid metabolism (CAM) that facilitates the uptake of carbon dioxide at night, improves its water-use efficiency under dry conditions. The succulent leaves collect and store water in the leaf axils, where it is absorbed by surrounding tissue or by aerial roots (Aragon *et al*, 2012). Due to these physiological and morphological features, the vegetative growth of pineapple is less affected by water stress and they prefer arid conditions. This may be the reason why the plants in temperature stress and water stress showed significantly higher plant height.

3.2.2 Leaf Length

The leaf length has shown an increasing trend pattern from week 0 to week 80. The highest leaf length was shown in the plants under the treatment of temperature stress but with no water stress. The lowest leaf length was shown in the plants under the treatment of temperature stress and water stress.

According to Figure 7 which shows the leaf length at week 80, the highest leaf length was shown in plants in the treatment of no temperature stress and no water stress and it was not significantly different from all other treatments. The lowest leaf length was shown in plants in the treatment with temperature and water stress and it was also not significantly different from all other treatments. It is a typical feature of bromeliads to be less affected by stresses usually, as they grow well in arid conditions. This may be the reason for not having any significant difference between treatments. It shows that there is no impact of water stress and temperature stress on leaf length of pineapple. According to the ANOVA, both factors such as temperature and water have not significantly influenced the variation of the leaf length and therefore the interaction effect was not significant.

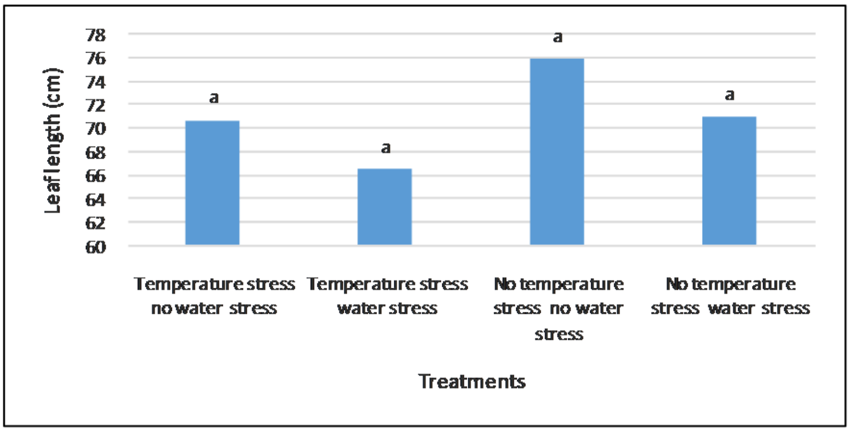

Fig. 7. Leaf Length after 80 Weeks

In agreement with previous results obtained in the same experimental system (Aragón et al. 2012), succulence, normally associated to leaf anatomy of CAM plants of different families (Madison, 1977), was also verified. Mesophyll tight cells with highly enlarged vacuoles contribute not only to water but also to malic acid storage (Winter and Smith, 1996).

3.2.3. Number of Leaves

Number of leaves has shown an increasing trend pattern up to the period of 64 weeks and then it has shown a decreasing trend pattern. The highest number of leaves was shown in the plants under the treatment of temperature and water stress. The lowest number of leaves was shown in the treatment of no temperature stress and no water stress.

According to Figure 8, the highest number of leaves at week 80 was shown in the treatment with temperature stress and water stress and it was significantly different from other treatments. The lowest number of leaves was shown in the treatment with no temperature and no water stress. However, this treatment was not significantly different from the treatment of no temperature stress but with water stress. As it is shown by Figure 7 the temperature and water stress have not exerted a negative impact on the number of leaves. According to the ANOVA both factors, temperature stress and water stress have not significantly influenced the number of leaves. Furthermore interaction effect was not significant.

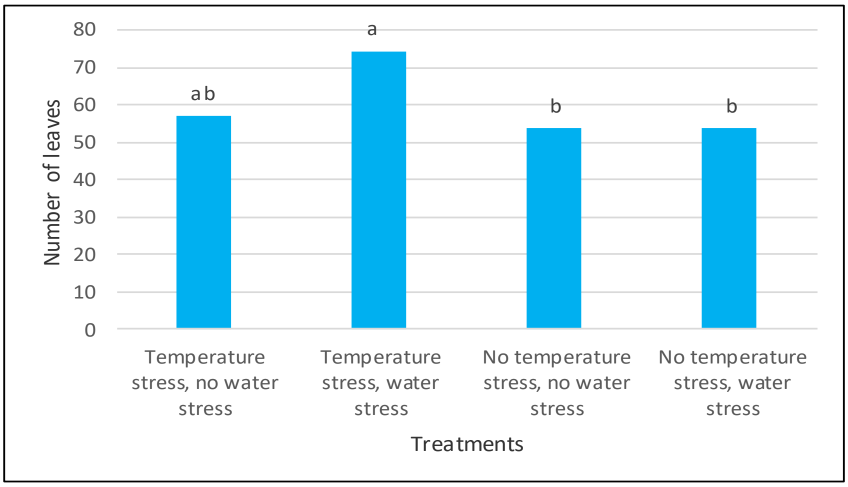

Fig. 8. Number of Leaves from Week 0 to Week 80

Pineapple being a plant exhibiting crassulacean acid metabolism and also having the ability to collect and store water in the rosette like leaf arrangement, and with the special features like the water-storing phytotelmata, the plant is able to adapt to water and heat stress (Lüttge, 2004). CAM physiological and biochemical features are deeply associated to leaf anatomy. In opposition to the majority of non- CAM plants, the anatomy of adult pineapple leaves is characterized by the presence of aerating canals, fiber strands, water storage tissue and hypodermis as described in adult pineapple plants as reported by D´Eeckenbrugge and Leal (2003) and more recently, by Pérez *et al.*(2012) in new pineapple somaclonal variants. Anatomy of *invitro* and *exvitro* pineapple leaves has also been compared (Barboza *et al*. 2006). Leaf anatomy of in vitro propagated pineapple plants also shows canals, fibers, an aquifer parenchyma, a chlorenchyma and hypodermic tissue, mostly maintained during the initial *exvitro* stages. However, the leaf anatomy of CAM plants has a high degree of plasticity in response to the environmental conditions

3.3 Yield Parameters

3.3.1 Fruit Diameter

Figure 9 indicated that the diameter of the fruit was maximum when the plants were under water stress and temperature stress. However, the diameter of the fruit was lowest in no temperature and no water stress which is significantly different from other treatments except the treatment with temperature stress but no water stress. High diameter observed with pineapple fruits seemed to indicate a gain of flesh. It would mean that fruits contain more juice, when fruits were grown under temperature and/ or water stress. It shows that pineapple prefer stressful condition due to its specialized physiological processes. When CAM plants are under stress, they produce large cells with water storage ability as a heat dissipation strategy which induces large diameter fruits than the fruits of plants growing under no stress conditions (Espírito and Pugialli, 1998). The large vacuoles of the cells of those tissues are also associated to malic acid storage, an aspect described in a number of CAM species.

According to the ANOVA both factors water stress and temperature stress and also the interaction effect have not significantly influenced on the diameter of the fruit. Therefore, there was no interaction effect.

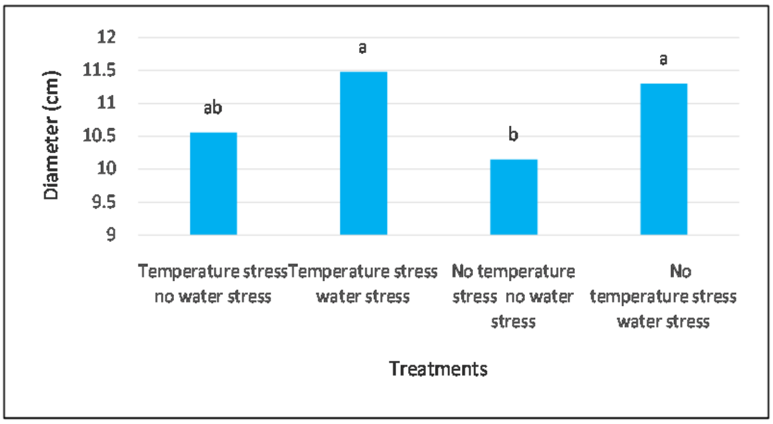

Fig. 9. Effect of Temperature Stress and Water Stress on Diameter of Pineapple fruit

3.3.2 Length of Fruits without Crown

According to Figure 10, the treatment with temperature stress and water stress has shown the highest length of fruit without crown which is significantly different from the length of fruit without crown in no temperature stress and no water stress. But the length of fruit without crown in temperature and water stress treatment is not significantly different from temperature stress and no water stress and no temperature stress with water stress. In the interest of the consumer what is important is the length of fruit not that of the crown. According to the ANOVA both factors such as water and temperature have not created any significant difference and also interaction effect has not imposed any significant influence on the length of fruit without crown. Therefore, there was no interaction effect.

Therefore, it can be inferred that the temperature and the water stress which was imposed on the pineapple plants have a positive impact on the length of the fruits. These finding of fruits with larger diameter and higher length than the fruits under no stress conditions agree with the finding of Espírito and Pugialli (1998). This study showed that pineapple would successfully cope up with global warming induced temperature stress and water stress.

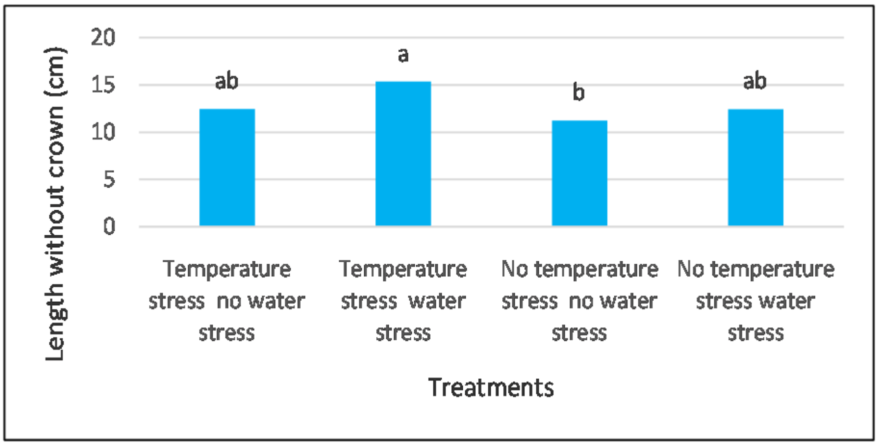

Fig.10. Effect of Temperature Stress and Water Stress on Length of fruit without Crown

3.3.3 Weight of Fruit without Crown

According to Figure 11, treatment with temperature stress and no water stress has shown the highest value for weight without crown. However, it was not significantly different from all other treatments. The treatment with no temperature and no water stress has shown the lowest weight without crown but it was not significantly different from all other treatments. This shows that temperature and /or water stress have not imposed any negative impact on weight of fruit without crown. Because they yield successfully, even in stressful situations they contribute to the world market.

According to the ANOVA, both factors water stress and temperature stress have not significantly influenced on the variation of the weight of fruit without crown. Also interaction effect has not created any significant difference on the weight without crown. Therefore, there was no interaction effect as the interaction effect was not significant.

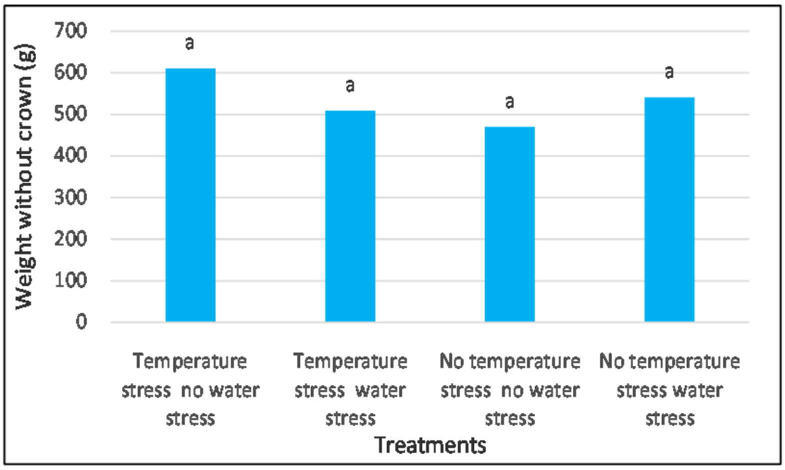

Fig.11.Effect of Temperature Stress and Water Stress on Weight of Fruit without Crown

Pineapple has unique anatomical and physiological modifications which enable the plant tosurvive periods of water stress. Under drought conditions, the plants are as hardy as cactus. For these and other reasons, the pineapple is one of the few crop plants that can be classified as a true xerophyte (Aragon *et al* 2012). In areas where water is limiting, the effects of water stress on the growth and yield of pineapple are of particular interest.

3.4 Quality Parameters of Pineapple

3.4.1 Firmness of the Flesh

According to Figure 12, the treatment with temperature stress and water stress has shown the lowest firmness of the flesh but it was not significantly different from all other treatments. The highest firmness of the flesh was shown in the treatment with temperature stress and with no water stress and it was also not significantly different from all other treatments. According to the ANOVA, temperature stress and water stress have not contributed to a significant influence for the firmness of the flesh. And also there was no interaction effect, because interaction effect was not significant.

This indicated that fruit is soft when the plant is under temperature stress and water stress. When firmness of flesh is low or when fruit is soft, it is preferred over the hard flesh fruits. Firmness of fruit is determined by the cell wall structure and cuticle properties (Chaib*et.al.*,2007). When the fruit is ripening there is cell wall degradation and remodelling of cell wall which causes softening of the flesh of the fruits (Matas*et.al,* 2009). When CAM plants are under stress, they produce large cells with water storage ability as a heat dissipation strategy (Espírito and Pugialli, 1998). When the pineapple fruits are exposed to high pre-harvest temperature a disorder with similarities to water core which is flesh translucence in pineapple occurs, where the symptoms are water soaking and increased porosity (Paull and Chen, 2000). Relatively high fruit temperatures early in the season may induce some tolerance to heat during later fruit growth; the disorder appears to be associated with heat stress in these later stages. This also causes the flesh of the pineapple fruits to be soft (Paull and Chen, 2000).

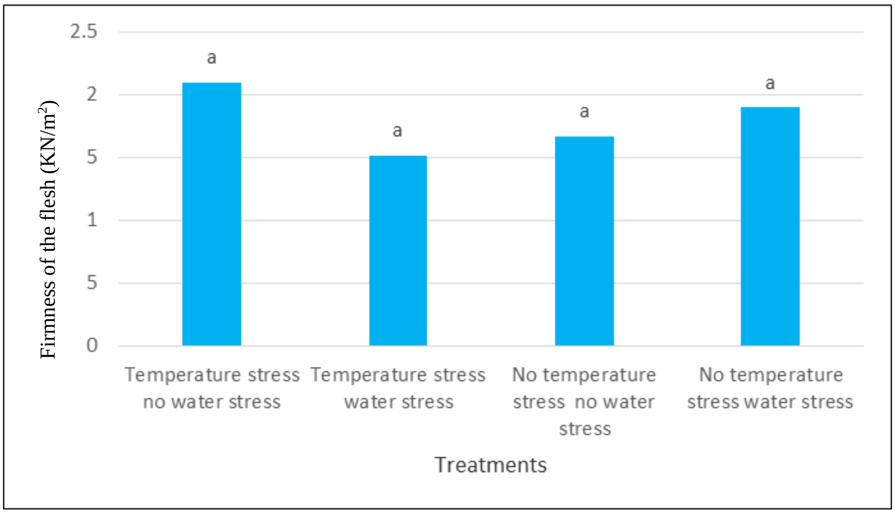

Fig. 12. Effect of Temperature Stress and Water Stress on Firmness of the Flesh

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3.4.2 pH of the Fruit

According to Figure 13, the treatment with temperature stress and no water stress has shown the highest pH value and it was significantly different from all other treatments. The treatment with no temperature and no water stress has shown the lowest pH value (3.1-3.2) and it was also significantly different from all other treatments. This shows that when the plants are under stress, they produce fruits having comparatively higher pH (3.7-4.3) compared to the fruits in no temperature stress and no water stress (3.1). Microorganisms have minimum and an optimum pH requirement for their growth. The excellent storing qualities of fruits are related to their respective pH, such as fruits with low pH value are usually not really spoiled by bacteria. According to the ANOVA, temperature stress was the only factor that contributed significantly on the pH. The other factor water stress has not contributed significantly on the pH. However, the interaction effect was significant, therefore, there was an interaction effect.

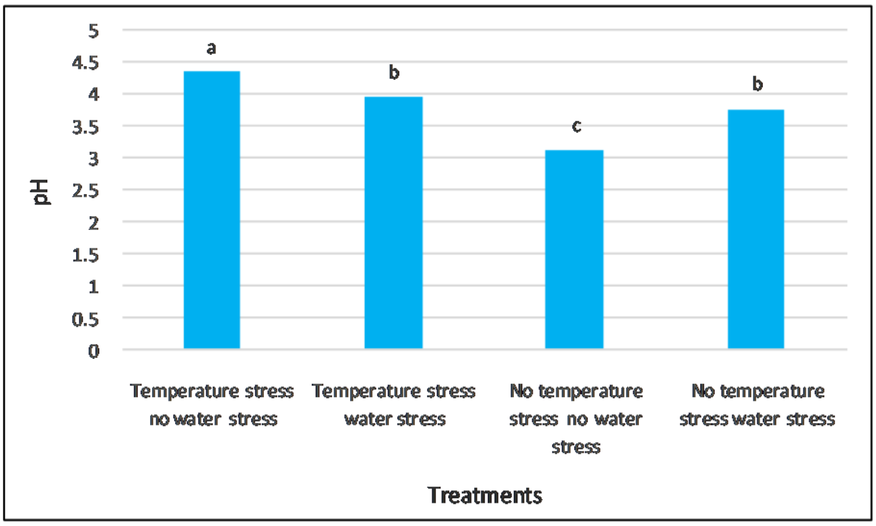

Fig.13.Effect of Temperature Stress and Water Stress on pH of the Pineapple Fruit

According to Andersen and Wilkins (1989) and Hummel *et al*, (2010) water stress increases the accumulation of organic acids in the leaves and xylem fluid which leads to import of organic acids to the fruit resulting in low pH. However, as reported by Esteban *et al*. (1999), des Gachons*et al*. (2005), and Thakur and Singh, (2012) there is a positive relationship between water supply and organic acid content in ripe fruits indicating a lower pH under no water stress condition.The daily variations of mesophyll pH in CAM plants usually follow the trend of values decreasing in the dark period due to the accumulation of malic acid and increasing in the light period when malic acid released from the vacuole is decarboxylated by cytoplasmic malic enzyme (Antony *et al*. 2008). In fact, it is possible to establish a direct relationship between the low pH values in the dark in vitro and ex vitro CAM induced plants and the high malic acid concentrations measured (Aragón *et al*. 2012).

3.4.3 Total Soluble Solids

According to Figure 14, the treatment with no temperature stress and no water stress has shown the highest value for TSS and it was significantly different from treatment with temperature stress and water stress but not significantly different from fruits from the treatments of temperature stress with no water stress and no temperature stress with water stress.

The treatment with temperature stress and water stress showed the lowest value for TSS. The total soluble solid is an indication of the amount of sugars present in the fruit. However, May (1993) has observed that low water stress resulted in products with best soluble solids in tomatoes. According to the ANOVA, both factors the water stress and temperature stress have not imposed any significant effect. Also interaction effect has not contributed any significant influence on the total soluble solids. Therefore, there was no interaction effect.

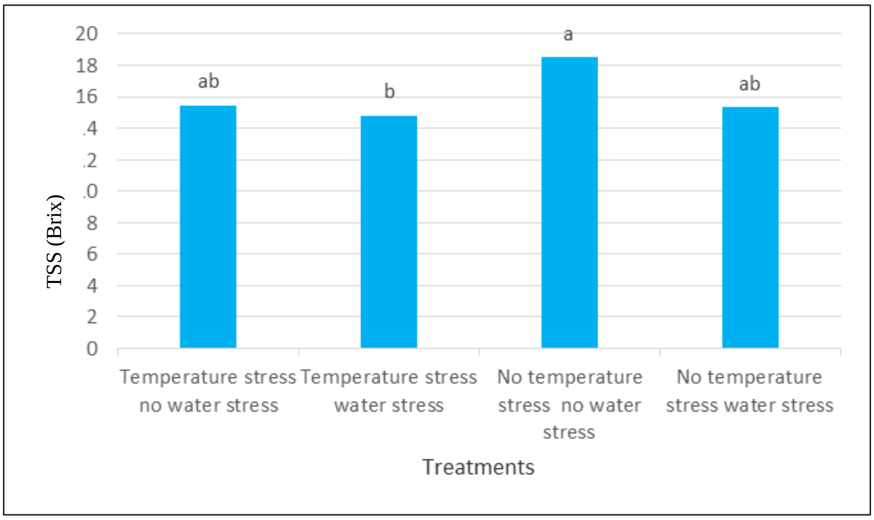

Fig. 14. Effect of Temperature Stress and Water Stress on Total Soluble Solids of Fruit

4 CONCLUSIONS AND RECOMMENDATIONS

The results obtained for pineapple propose that the vegetative growth was not affected much due to temperature stress and water stress imposed, reflecting global warming. The vegetative growth parameters such as plant height, number of leaves and leaf length also have not been affected significantly by the temperature stress and the water stress imposed. This suggests that the vegetative growth of *in vitro* propagated pineapple variety Kew would be less affected by the predicted global warming.

As pineapple is a plant exhibiting heat dissipation strategies, the diameter of pineapple fruit was significantly high under both temperature stress and water stress. It shows that temperature stress and water stress have a positive impact on the diameter and size of the fruit. The length of fruit without the crown also has shown a positive results when the temperature stress and water stress were imposed. The weight of fruit without the crown also has not shown a considerably significant reduction due to stresses imposed. Therefore, it can be inferred that yield of pineapple variety 'Kew' would be increased considerably, on exposure to the temperature and water stress due to the predicted climate change.

According to the quality parameters of pineapple, the firmness of the flesh has shown a comparatively low value, when the plants were under stress, therefore the flesh is soft which could be preferred by the consumers. Hence, it is evident from these results that the plant would produce fruits that would be preferred by the consumers, when it is under temperature and water stress. When the plants were under stress, the pineapple fruits that they produced showed a acidic pH (3.7- 4.3) which is one of the good post-harvest qualities as there will be less spoilage by bacteria. The total soluble solids of fruits showed a slight decline when the plants were under stress but that is not much different from the fruits produced without any stress.

The finding of this study indicates the pineapple will be a successful crop even if there is temperature and water stress due to global warming. Therefore, farmers are encouraged to cultivate pineapple even under temperature and water stress conditions to increase the yield without crop failures as experienced in other crops.

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