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In vivo screening of salinity tolerance in Giant Swamp Taro *(Cyrtosperma merkusii)*

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Abstract

Giant Swamp Taro (Cyrtosperma merkusii) is a staple food crop in the Pacific, especially in the low lying atoll islands such as Tuvalu and Kiribati. This is owing to its ability to survive under poor soil conditions and harsh environments. However, as a result of the effects of climate change such as sea water inundation and intrusion into the fresh ground water lens, this crop is now under threat. To address this issue an adaption approach was taken whereby, Cyrtosperma merkusii was screened in vivo for salt tolerance. The epistemology followed random selection of two cultivars Ikaraoi and Katutu. These two cultivars were subjected to 0% (0 parts per trillion), 0.5% (5 ppt), 1% (10 ppt), 1.5% (15 ppt) and 2% (20 ppt) of salt in Yates's advance seedling common potting mix. Both cultivars were able to tolerate salinity levels up-to 5ppt which is significantly more than the salt tolerance in glycophytes of 2.83 ppt. This research provides an insight into the variation of salt tolerance that may exist in C.merkusii gene pool, which can be used to adapt to natural disasters and buffer its impacts.

Keywords*: Giant Swamp Taro (Cyrtosperma merkusii), Climate change, Salt tolerance*

1. Introduction

Sea level rise is a silent natural disaster that results in an increase of salinity levels in the groundwater lens. This natural disaster may not be as obvious as hurricanes and cyclones but its impacts can render atoll islands becoming unproductive. Groundwater lens forms due to a delicate balance between the rain fed fresh water and salty seawater. It is the main source of water supply that supports atoll vegetation. Climate change related sea level rise greatly threatens this ground water lens by means of salt water intrusion and sea water inundation, both of which result in increased groundwater salinity levels (Dunn, 1976; Woodraffe, 1989 & 2008; White *et al*, 1999; Webb, 2007; White and Falkland, 2010). This increase in turn, threatens the food security of the atoll islands as crop production which is greatly reduced. One such crop that is a staple food on these atolls is Giant Swamp Taro (*Cyrtosperma merkusii)*. This particular aroid is known for its ability to survive harsh atoll environments. However, it is currently also threatened by the increased salinity levels in the ground water lens (White *et al*, 1999; Webb, 2007; White and Falkland, 2010). The loss of this aroid would not only mean reduced food security on the atolls, but also a loss of their identity. As giant swamp taro over the years has intertwined into the cultures and traditions of the Pacific islanders.

The development of a screening methodology for salinity tolerance included the evaluation of different salinity levels, using Artificial Sea Water (ASW) to mimic the effects of sea water inundation and ground water lens intrusion. This experiment was conducted on two groups of Giant Swamp taro cultivars *(Cyrtosperma merkusii)* from Kiribati, namely the *'*Ikaraoi*'* and the *'*Katutu*'*.

2. Methodology

These cultivars Ikaraoi that is larger, has longer maturity period and has less number of suckers and Katutu which matures faster, is shorter in size and has many suckers were obtained from the Secretariat of the Pacific Community (SPC), Centre for Pacific Crops and Trees (CePaCT). These accessions were imported by CePaCT from Kiribati in 2009. Plants were subjected to five different levels of salinity, namely 0.5% (5 ppt), 1.0% (10 ppt), 1.5% (15 ppt), 2.0% (20 ppt) plus the control 0% (0 ppt) salt. Each treatment was replicated five times thus a total of 50 plants were used. Each of the 50 plants were individually potted in 10 × 10cm black pots which were standing in trays to avoid run-off of the applied salt solutions. 250 mL of Yates's advance seedling common potting mix was used as the planting medium. Tissue cultured plants were transferred into the pots using CePaCT's procedure whereby the plants were gently washed with tap water to remove the growth medium from the plant, ensuring that no part of the plant is damaged. The plants used in this *in vivo* experiment were at least 6cm in size from the base of the stem to the apex of the tallest leaf when removed from the tissue culture bottles. The plants were firmly planted in the pots and a clear plastic bag was used to cover the plants to allow acclimatization to the environment, prevent shock and dehydration. Plastic bags were removed progressively over a one month period. For example plastic bags were removed for an hour the first day, the following day it was removed for two hours and so on until it was completely removed. Plants were watered with 40 ml of tap water three times a week. From the time of transfer into the pots, the plants were kept in a shaded green house in the CePaCT at approximately 25$\pm$2ºC

and after three months transferred to a typical green netted screen house at approximate temperature of 27±2 ºC. In this screen house plants were watered with 50ml of tap water three times a week. Plants were allowed to acclimatize for another month in this screen house, and then 50 ml of ASW salt solution was applied on an incremental basis of 0.5% salt per week for four weeks. The experiment was carried-out for one month after the five months of transfer and acclimatization phase of the plants.

The effects of the treatments were assessed mainly on the morphology of the plant except for the chlorophyll content measurement. The morphological traits assessed were number of leaves emerging, root size and number of leaves dying. These traits were measured weekly once a week at the end of each week. The chlorophyll content was measured at the end of the experiment.

The criteria used for counting an emerging leaf was when a leaf's full leaf blade length (though not fully opened) became visible. When visually estimated leaves displayed 50% chlorosis, the leaf was counted as dead.

2.1 Chlorophyll Content

After the month of exposure to the various treatments, leaves were analysed for chlorophyll content using method described by Kreast and Grant (1976). One gram of leaf was soaked for 2 minutes in 5 ml of 80% acetone (80 ml acetone plus 20 ml distilled water). This was done to soften the leaves, for easy extraction of the chlorophylls. After two minutes the acetone was drained and the leaves were grounded using a mortar and pestle with 2 ml of 80% acetone. The extracted juice of 1.5 ml was then centrifuged in a micro centrifuge at 8000 revolutions per minute (rmp) for 15 minutes at 5 °C. The supernatant was then transferred using a micropipette into a cuvette. The chlorophyll content was determined by measuring absorbency at two wavelengths Abs 664 and Abs 647 in spectrophotometer. The spectrophotometer was first loaded with the blank or controls (80% acetone) then the other supernatants were loaded and readings recorded. After each reading the cuvettes were rinsed with 80% acetone twice and once with the next supernatant that was to be loaded, this prevented contamination of the supernatants from the residue left in the cuvettes. Using the absorbance readings, chlorophyll content was calculated.

The data was analysed using the Genstat statistics software. The total plant response and the two cultivar group response were compared against the five salinity levels using ANOVA. For the *in vivo* a percentage survival analysis was also done looking at the number of plants that were alive when the experiment ended.

3.0 Results

Table 1. Pre and post experiment salinity levels.

Pre salinity (%)	Post salinity (%)
0	0
0.5	1
1	1.5
1.5	1.5

Salinity levels in the pots subjected to 0.5% salt and 1.0 salt had increase in salinity levels, while those for the control and the 1.5% salt which remained the same.

Table 2. Percentage survival rate of Ikaraoi and Katutu.

Cultivar group	Salt (%)	No. alive	No. dead	Survival (%)
Ikaraoi	0	5	0	100
	0.5	3	2	60
	1	0	5	0
	1.5	0	5	0
Katutu	0	5	0	100
	0.5	3	2	60
	1	0	5	0
	1.5	0	5	0

The cultivars also have the same survival response to the subjected salinity levels with 100% survival at 0% salt and 60% at 0.5% salt and 0% survival for salinity levels higher than 0% salt.

Table 3. Percentage survival rate of the various salinity levels.

Salt (%)	No alive	No. dead	Survival (%)
0	10	0	100
0.5	6	4	60
1	0	10	0
1.5	0	10	0

All the plants subjected to 0% salt survived and those at 0.5% salinity had a percentage survival is of 60%. While those subjected to higher salinities died such 1.0 % salt and 1.5% salt giving a percentage survival rate of 0%.

Table 4. Ikaraoi and Katutu response to the salinity levels with significance value *f*.

Parameter	*f*	Ikaraoi	Katutu
No. Leaves	0.437	2.23±0.69	2.28±0.71
Root (cm)	1	1.750±0.47	1.75±0.47
No. Dying leaves	0.681	3.9±0.79	3.7±0.82
Chlorophyll (µg/ml/g)	0.828	20.26±1	22.26±1

Both the cultivars Ikaraoi and Katutu have no significant difference in their response of number of leaves emerging, root size, number of leaves dying and chlorophyll content to the subjected salinity levels with all the *f* values being more than *0.05*.

Table 5. Plant response to the various salinity levels with significance value *f*.

Parameter	*f*	0%	0.50%
No. Leaves	0.04	2±0.47	2.5±0.85
Root (cm)	0.002	2±0.47	1.5±0.53
No. Dying leaves	0.001	1.6±0.52	6±0.47
Chlorophyll (µg/ml/g)	0.863	19.48±1	23.05±1

The combined response of the cultivars shows that, there is a significant difference in response of the number of leaves emerging, root size and number of leaves dying to the two salinity levels of 0% salt and 0.5% salt with *f* values less than 0.05. While there is no significant difference seen in chlorophyll content with *f* values more than 0.05.

4. Discussion

After a successful acclimatization phase from the second week of the incremental phase plants started to wilt with heavy chlorosis of leaves. By the third week of the increment phase plants started to die out. This allowed only three increments to occur, the highest being 1.5% salt. Therefore the final salinity levels tested *in vivo* were 0%, 0.5%, 1.0% and 1.5% salt. Since the plants died out, only the number of leaves emerging, rooting, number of dying leaves and chlorophyll content were measured.

Conducting experiments *in vivo* does not allow control over the environmental factors such as temperature, light, heat and moisture leaving plants exposed to the elements of nature (Munns and James, 2008). Water loss due to evaporation and transpiration is one of the factors that might have resulted in the low survival rate of the plants in the green house. Evaporation and transpiration reduces the amount of water in the pots and moisture in the soil, resulting in an increase in the initial salt concentrations. Table 1 reports the salinity levels measured in the pots at the beginning and end of the experiments which showed a significant increase in the salt concentrations from the applied 0.5% to 1%, from the applied 1% to 1.5% salt.

Furthermore, in the exposed nature of an *in vivo* system the air spaces within the soil provided a greater surface area to volume ratio for evaporation to take place. All of these factors not only add on to water loss from plant and soil, but also affect the physiological mechanisms operating to maintain a plant's ionic and osmolic homeostatis (Zhu, 2001).

The control treatment (without any salt) had 100% survival rate. The plants had successfully acclimatized in the control treatments. Hence salinity application before the acclimatization period of plants could be ruled out as a cause of the low survival rate. After the application of the three salinity levels plant health started to deteriorate, as can be seen by the 60% survival rate in 0.5% salt and the zero percent survival rate of 1.0% and 1.5% salt (Table 2). This was the same for both the cultivars and when combined the same results were achieved for the overall plant response (Table 3).

4.1 Cultivar Group Response

Since the plants of the 1.0% and 1.5% salt had died out analysis was done on the control as "no salt" to the 0.5% salt as "salted". Comparison of the two cultivar groups showed that they both had the same response to the applied salinity levels of 0% and 0.5% salt (Table 4). There was no increase in corm size or production of suckers in any of the treatments for either of the cultivars in the three weeks, thus these parameters were not evaluated. Of the four evaluated parameters of number of leaves, rooting size, number of dying leaves and chlorophyll content, none recorded *f* probabilities less than 0.05. Indicating that, none of the evaluated parameters of the two groups of cultivars have any significant difference. Both had the same response to the salinity levels tested. Similar response was also seen in their rate of survival when subjected to the increased levels of salinities (Table 2) (Munns and James, 2008).

4.2 Plant Response

With an *f* probability of less than 0.05, rooting size, number of leaves emerging and number of leaves dying had significant differences in the response to the two salinity levels. Number of leaves emerging and number of leaves dying increased with increase in salinity, while rooting decreased. Roots of plants in 0% salt conditions were fibrous, healthy and well developed, while the roots of plants in the 0.5% salt had died/melted out around the root tips. However, the majority of the root system was alive and healthy. There was no significant difference in the chlorophyll content with an *f* probability of more than 0.05. In plants subjected to 0.5% it was seen that while old leaves wilted and died, new leaves had sprouted. Furthermore, despite the wilting of old leaves the chlorophyll content of the new leaves was the same as the control plants, with a probability of similarity at 0.863 (Table 5).

Wilting of leaves indicated that the plants had experienced the first stress, the osmotic stress; this indicative response is consistent with many other experiments such as the work done by D'antonio and Weber (1999), Liska *et al*. (2004), Aghaeri (2008) and Kchaou *et al*. (2010). Following the osmotic stress, ionic stress takes its toll resulting in early senescence of old leaves (*f* 0.001). However, at 0.5%

salt some level of ionic and osmotic homoeostasis might have been obtained resulting in new shoots (*f* 0.04). These positive responses to the salt levels tested has also been seen in salt tolerance screening of salt tolerant plants by Fatokun *et al*. (2002) in cowpeas, in wild einkom wheat by Yesayan *et al*. (2008) and Colmer *et al*. (2006). Thus, it can be said that the two cultivars Ikaraoi and Katutu cannot survive salinity levels of more than 0.5% (5 ppt) of ASW *in vivo*. Gylcophytes are able to tolerate 0.283% (2.83 ppt) of salinity (Flowers, 2003), which is much less the 0.5% (5 ppt) salinity obtained by the two cultivars. This shows the potential *C. merkusii* has the potential to be developed as a salt tolerant crop.

5.0 Conclusion

Giant swamp taro a local food crop of the Pacific and an everyday food source for the atoll islands, is also a neglected and underutilized crop species in the region. Coupled with the effects of climate change, giant swamp taro is threatened through loss of its diverse range of cultivars and traditional cultivation knowledge. This research revealed that the two groups of cultivars Ikaraoi and Katutu could tolerate upto 0.5% or 5ppt of salinity, which is significantly more than the 2.83ppt tolerance of glycophtyes. In addition, in an *in vivo* system plants are exposed to variable temperature, light intensity, photoperiod duration, humidity, heat and wind. This exposure causes unaccounted increase in salinity as soil and plant water potential drops. The methodology used gives a strong foundation for salt tolerance screening and is practical enough for the Pacific where there is lack of technical and financial resources. However, there is still a lot of improvement that needs to be done, especially to account for the increase in salinity due to exposure. The methodology can be improved by using larger sample populations and more cultivars. Also constant monitoring of soil salinity levels in an *in vivo* systems to avoid unaccounted increases in applied salinity levels. Hence, paving the way for further research in salt tolerant giant swamp taro cultivars and food security in the atoll islands.

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