

RESEARCH ARTICLE

Effects of fertilization levels on growth performance of micro-propagated *Cryptocoryne wendtii* (Water Trumpet)

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Abstract: This study was conducted to evaluate the effects of different fertilization levels on growth performance of micro propagated water trumpet, *Cryptocoryne wendtii* to find the most suitable hardening medium. Micro-propagated ninety-six individual plants potted in the commonly used substrate of coir particles were used for the experiment and subjected to different fertilization levels of Albert's solution. Plants were potted just after being taken from the vessels and eight plant pots kept in the tanks (25.5 L water) containing 0.5 gL⁻¹, 1.0 gL⁻¹, and 2.0 gL⁻¹ of Albert's solution, and fertilizer was not added into the control. Each treatment and control had three replicates and the experiment was conducted for nine weeks under natural light and temperature conditions. At the end of the experiment, plant growth performance in terms of plant height, root height, number of leaves etc. and water quality parameters were compared in One Way Analysis of Variance using SPSS (16.0). The electrical conductivity (EC) of the 0, 0.5, 1.0, 2.0 gL⁻¹ treatments were 0.075, 0.75, 1.5, 3.0 μS/m, respectively. At the end of the experiment, plants grown in 0.5 gL⁻¹ showed the best growth performance giving significantly highest plant height (11.77 ± 0.40 cm), root height (5.77 ± 0.03 cm), number of leaves (10.0 ± 0.3), number of roots (8.3 ± 0.3), wet weight of whole plant (0.3180 ± 0.0200 g), dry weight of whole plant (0.0206 ± 0.0003 g) and the largest leaf area (2.54 ± 0.01 cm²) in compared to other two treatments and control. In contrast, the uptake of Albert's solution significantly increased with the increasing EC level. The results of this study revealed that, 0.5 gL⁻¹ Albert's solution is the most suitable fertilization level among tested three fertilization levels for the hardening process of micro-propagated *C. wendtii*.

Keywords: Albert's solution, *Cryptocoryne wendtii*, Fertilization level, Growth performance, Hardening

Introduction

Over the past decade, Sri Lankan aquatic plant sector has been developed with the escalating demand from developed countries (Bambaranda & Peiris, 2016). Among them, *Cryptocoryne* is one of the endemic genera that is on sale as popular aquatic plant (Dissanayake et al., 2007). It is an amphibious genus of Araceae family. Out of fifty species, fourteen are endemic to Sri Lanka including *C. wendtii*. Due to the special applicability for water gardening *C. wendtii*, it

secures high demand in both local and export market mainly from developed countries (Yapabandara & Ranasinghe, 2007). Therefore, *Cryptocoryne* is illegally collected and harvested from the wild for international trade. Due to high market demands, people tend to exploit this plant from the natural environment (Dissanayake et al., 2007). Thus, *C. wendtii* is declared as a critically endangered species in the Red List of International Union for the conservation of Nature (IUCN, 2012).

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Artificial *in vitro* micro-propagation is a method developed to address the problems of inadequate supply of plantlets for aquaculture (Yapabandara & Ranasinghe, 2007). But, wide use of micro-propagated plants is restricted by a high percentage of plant losses or damaging in the period of transformation to the *ex vitro* condition due to lack of knowledge on the required nutrient level for the plant (Deb & Imchen, 2010). This is a grey area in the field of aquatic plant culture. Therefore, suitable fertilization levels for *ex vitro* cultivation of high demanding aquatic plant should be investigated.

However, the electrical conductivity (EC) is an indirect suggestion of the nutrient level of the culture medium (Samarakoon et al., 2006). Total ion amount of dissolved fertilizers or salts origins an osmotic pressure as a colligative property of nutrient solution (Trejo-Téllez & Gómez-Merino, 2012). EC value causes nutrient uptake level through this osmotic pressure. (Trejo-Téllez & Gómez-Merino, 2012).

Therefore, this study was designed to evaluate the effects of fertilization levels on growth performance of micro propagated water trumpet in order to find out the most suitable nutrient medium for the acclimatization before being released to the natural ecosystem or export market.

Materials and Methods

The experiment was conducted under the natural light at the room temperature. *Cryptocoryne wendtii* was grown in glass tanks in a plant house premises at National Aquatic Resources Research and Development Agency (NARA). The study was carried out in 12 glass tanks (25 cm x 30 cm x 60 cm) filled up with 2/3 of de-chlorinated tap water (25.5 L). Each treatment had three replicates and tanks were randomly allocated for the treatments. Ninety six plantlets with approximately similar heights (10.13 ± 0.71 cm) and weights (0.155 ± 0.00 g) were randomly potted in 96 of plastic mesh pots (4.5 cm x 4.5 cm) using acid removed coir particles as the potting media.

Plant pots were arranged in two rows each with four pots in one row at the bottom of each tank. The plants were provided with four types of nutrient levels (0

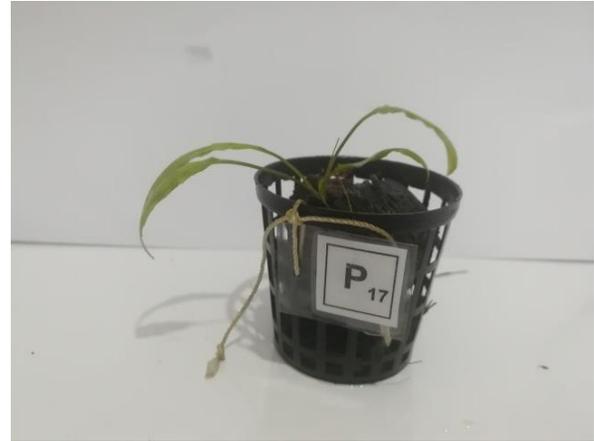


Plate 1: Plant potting design

gL^{-1} , $0.5 gL^{-1}$, $1.0 gL^{-1}$, $2.0 gL^{-1}$) of Albert's solution designated as C, 0.5A1, 1.0A1 and 2.0A1 respectively. Each individual plant pot was labeled properly, and eight plants were placed in a single glass tank. The reduction of water via evapotranspiration was controlled by adding deionized water and complete water renewal with correct fertilization level was done at three weeks intervals. Plant height, number of leaves and leaf area were measured every two weeks and root length, number of roots, wet and dry weights of plants were measured only at the beginning and at the end of the experiment. Weights were measured using mettler PJ 3600 top loading electronic balance and the leaf area was measured by using Image J latest version, Java application for scientific image processing (2017).

Temperature in the water and light intensity of each tanks were measured daily while pH, Dissolved Oxygen (DO), salinity, Electrical conductivity (EC) were measured weekly using the environmental multi- probe, YSI™ Model 556 Multi probe. The Nitrate (NO_3^-) and Phosphate (PO_4^{3-}) levels in water were measured at three weeks intervals. Relative growth rate (RGR) of plants and survival rate (%) were compared at the end of the experiment.

Specific growth rate (SGR)

$$\text{Specific growth rate} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Where,

\ln = Natural log

W_1 = Dry weight of plant recorded at time t_1

W_2 = Dry weight of plant recorded at time t_2

t_1 and t_2 were the interval of time respectively.

Plant survival rate (%)

$$\text{Plant survival rate (\%)} = \frac{\text{number of remaining plants} \times 100}{\text{number of plants originally}}$$

Nutrient use efficiency in the nutrient solution

$$\text{Apparent recovery efficiency} = \frac{\text{nutrient taken up by the plants (g)}}{\text{Nutritent applied (g)}}$$

Statistical Analysis

The growth performance of *C. wendtii* was compared in One Way Analysis of Variance (ANOVA) at the 5% significance level ($p < 0.05$). Duncan’s multiple

range test was used to detect the differences among treatment means. All statistical analysis were carried out using SPSS statistical software (SPSS 16.0).

Results and Discussion

Different fertilization levels were significantly affected on the growth performance of water trumpet (Fig 1, 2, 3 and 4, & Table 1). Significantly the highest growth performance was observed from 0.5 Al Solution considering plant height, root length, number of leaves, number of roots, leaf area, total wet weight and dry weight. At the end of the experiment, number of survived plants of the 0, 0.5, 1.0, 2.0 gL^{-1} treatments were 6.8 ± 0.3 , 7.2 ± 0.2 , 5.4 ± 0.1 , 4.6 ± 0.4 respectively.

Both wet and dry weights of different parts of plants (Fig 1) in 0.5 Al treatments showed significantly highest value followed by control and lowest by 2.0 Al treatments.

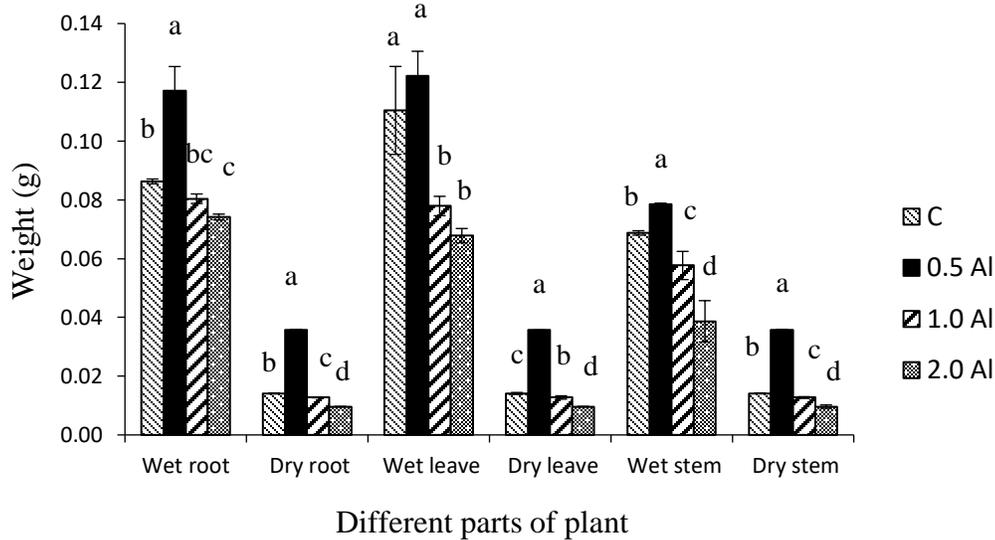


Figure 1: Variations of the means (\pm SD) of dry and wet weights of roots, leaves, and wet and dry stems of *C. wendtii*. (C-Control; 0.5 Al-0.5 gL^{-1} of Albert’s solution; 1.0 Al-1.0 gL^{-1} of Albert’s solution; 2.0 Al-2.0 gL^{-1} of Albert’s solution) ($n=24$)

Plant height suggested a sudden decrease in the elementary period of replantation in all treatments due to the loss of older leaves (Fig 2). After 14 days plants showed a regular growth performance.

The number of leaves also showed a sudden decrease in the pre-acclimatization period for all plants. After 14 days, only young leaves were remained in all plants (Fig 3).

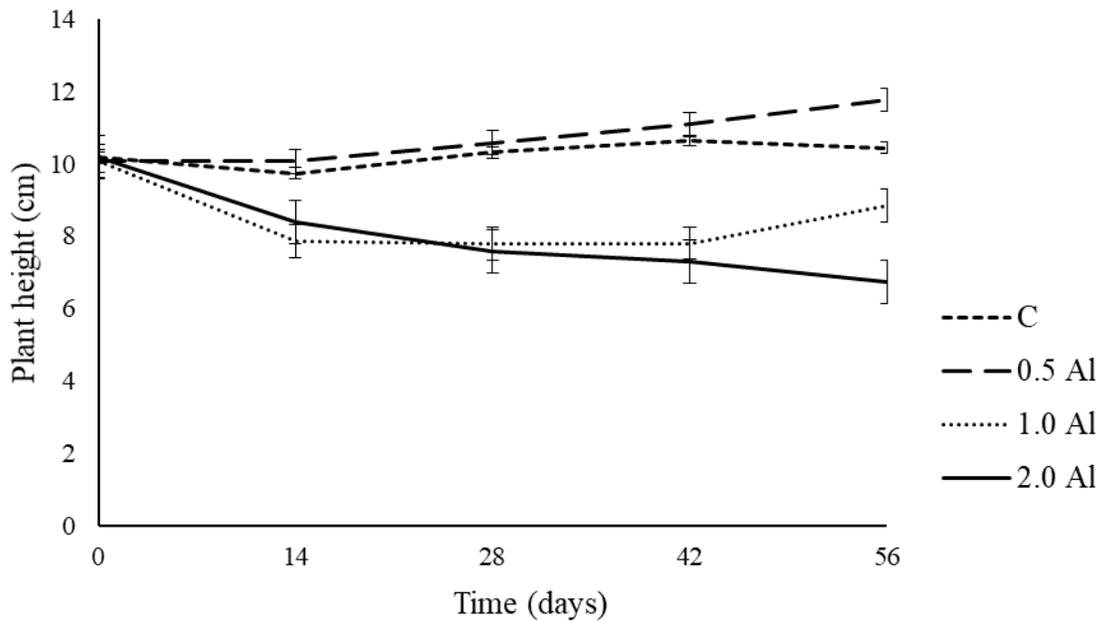


Figure 2: Variations of the means (\pm SD) of plant height in different levels of Albert’s solutions (C-Control; 0.5 Al-0.5 gL⁻¹ of Albert’s solution; 1.0 Al-1.0 gL⁻¹ of Albert’s solution; 2.0 Al-2.0 gL⁻¹ of Albert’s solution) (n = 24)

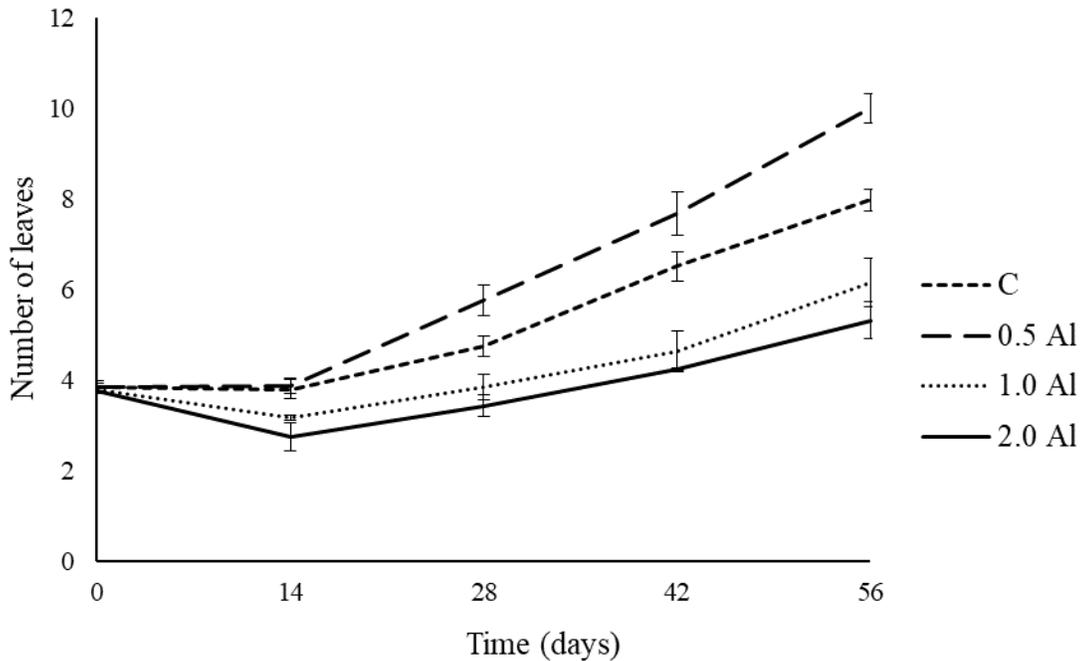


Figure 3: Variations of the means (\pm SD) of number of leaves in different levels of Albert’s solutions (C-Control; 0.5 Al-0.5 gL⁻¹ of Albert’s solution; 1.0 Al-1.0 gL⁻¹ of Albert’s solution; 2.0 Al-2.0 gL⁻¹ of Albert’s solution) (n = 24)

Excess nutrient symptoms were initially appeared on both older leaves and younger leaves. It depends on

the way of metabolism with respect to the nutrient mobilization of plants. If the plant is not able to breakdown the stored nutrients in mature leaves to

transport for young leaves, the nutrient imbalance symptoms were firstly appeared on younger leaves and growing points (Miyasaka et al., 2002). If the plant can breakdown stored nutrients in mature leaves, those are transport to the younger leaves and thus the symptom shows in older leaves. In this study, *Cryptocoryne wendtii* could breakdown nutrients in older leaves to transport for younger leaves. That is the reason for loss of older leaves in the period of pre-acclimatization period.

Pospóšilová et al., 1999 reveals that plants that just after taken from vessels contained a less developed cuticle in leaves. Also, in the time of plant introducing to the hardening condition, the tissue cultured plantlets have thin epicuticular waxes in leaves (Pospóšilová et al., 1999) and therefore, micro-propagated plants are very sensitive to a new environment. The exposure of leave tissue cells for a new environment cause for a loss of leaves or affect for a slow rate of leaf initiation. Hence, the number of leaves show a sudden decrement due to the higher rate of absorbing nutrients from all treatments.

Table 1: Plant Growth Parameters

Growth parameter	C	0.5 Al	1.0 Al	2.0 Al
Final Root Length (cm)	5.48 ± 0.05 ^b	5.77 ± 0.03 ^a	5.50 ± 0.10 ^b	4.00 ± 0.06 ^c
Final Number of roots	6.2 ± 0.0 ^b	8.3 ± 0.3 ^a	5.6 ± 0.3 ^c	5.4 ± 0.0 ^c

Mean ± SD within the same raw with different superscripts are significant different (p < 0.05) (C-Control; 0.5 Al-0.5 gL⁻¹ of Albert’s Solution; 1.0 Al-1.0 gL⁻¹ of Albert’s solution; 2.0 Al-2.0 gL⁻¹ of Albert’s solution) (n=24)

The plants in 0.5 Al Solution were showed an excellent growth curve, S shape like a growth curve of healthy plant (Fig 4). But the curve was not reached to the constant level during the study period.

Cao et al., 2019 suggested the Sigmoidal Growth Curve of a healthy plant. Thus, it was suggested that the plants have not reached to the complete maturation within this experimental time period.

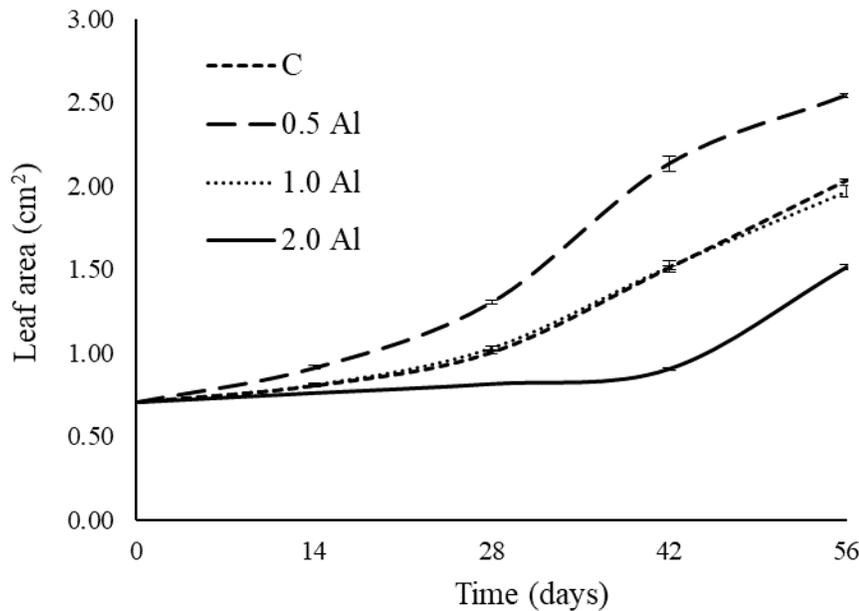


Figure 4: Variation of the means(±SD) of leaf area of water trumpet in different levels of Albert’s solution during the experimental period (C-Control; 0.5 Al-0.5 gL⁻¹ of Albert’s solution; 1.0 Al-1.0 gL⁻¹ of Albert’s solution; 2.0 Al-2.0 gL⁻¹ of Albert’s solution)

In this study, the electrical conductivity (EC) sequence is 0.075, 0.75, 1.5, 3.0 $\mu\text{S}/\text{m}$ consisting of both higher and lower EC values. Higher EC values cause to increase the osmotic pressure as a colligative property of nutrient solution (Samarakoon et al., 2006).

As there was no evapotranspiration, all the variation of EC level is depended on the absorption of nutrients

by plants. The ratio of nutrient uptake and the amount of nutrient applied were 0.26:1.28, 3.14:12.75, 17.44:25.5 and 39.59: 51.00 in plants grown in Control, 0.5 Al, 1.0 Al and 2.0Al treatments, respectively. The nutrient uptake increased in a concentration dependent manner while making an excessive uptake (Samarakoon et al., 2006).

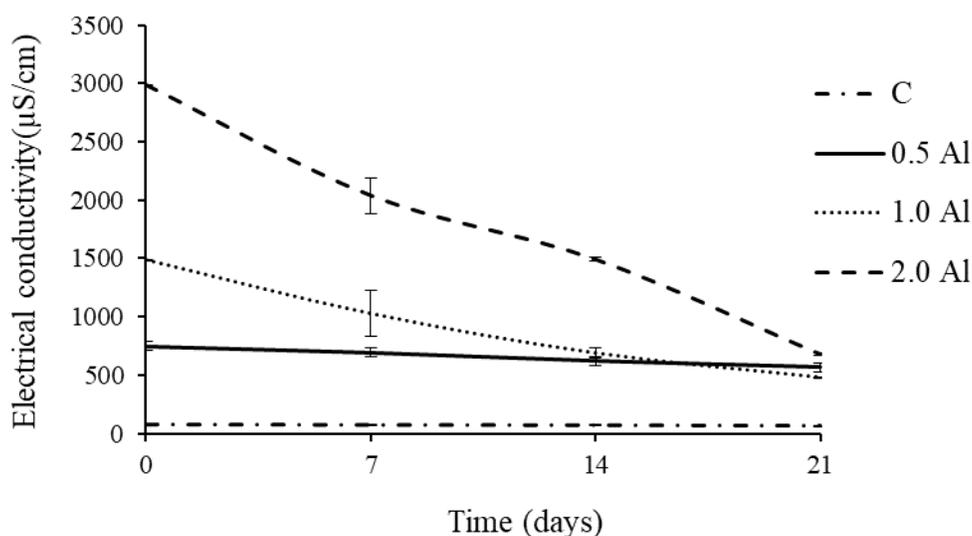


Figure 5: Variations of the mean±(SD) of electrical conductivity($\mu\text{S}/\text{cm}$) of different treatments within 21 days. (C- Control; 0.5 Al-0.5 gL^{-1} of Albert’s solution; 1.0 Al-1.0 gL^{-1} of Albert’s solution; 2.0 Al-2.0 gL^{-1} of Albert’s solution)

Plants with a relatively slow growth rate showed a low survival rate whereas plants with relatively high growth rates showed a high survival rate (Table 2).

The apparent recovery efficiency (ARE) values were significantly increased with the Albert’s solution concentration.

Table 2: RGR (Relative growth rate, gweek^{-1}), ARE (Apparent recovery efficiency) and Survival rate (%) of different treatments.

Parameter	C	0.5 Al	1.0 Al	2.0 Al
RGR	0.038 ± 0.003 ^b	0.124 ± 0.074 ^a	0.027 ± 0.007 ^b	0.004 ± 0.006 ^b
Survival Rate (%)	91.67 ± 7.22 ^a	100 ± 0.00 ^a	79.17 ± 7.22 ^b	70.83 ± 7.22 ^b
ARE	0.20 ± 0.00 ^d	0.25 ± 0.00 ^c	0.68 ± 0.00 ^b	0.77 ± 0.00 ^a

Mean ± SD within each row followed by superscript letter (a,b,c,d) are significant difference ($p < 0.05$). (C-Control; 0.5 Al-0.5 gL^{-1} of Albert’s solutions; 1.0 Al-1.0 gL^{-1} of Albert’s solution; 2.0 Al-2.0 gL^{-1} of Albert’s solution)

Negative correlations were observed between RGR and ARE (Fig 6), and between survival rate and ARE (Fig 7). There was a positive correlation between RGR and the survival rate (Fig 8). Though uptake amount of Albert's solution by plants was increased in parallel to the exposure nutrient level, it has not affected for increase the growth performance because of the nutrient imbalance in higher fertilizer concentrations. The nutrient solution increases its concentration due to high water absorption in high concentrated solutions.

The effects of temperature, light intensity, pH and Dissolved Oxygen (DO) levels of different treatments were not statistically significant. But the values of salinity, nitrate (NO₃⁻) and phosphate (PO₄⁻³) concentrations were statistically different.

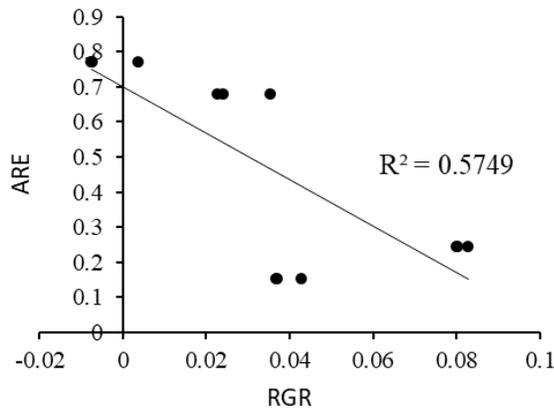


Figure 6: Correlation between RGR (Relative growth rate) (g g⁻¹week⁻¹) and ARE (Apparent recovery efficiency).

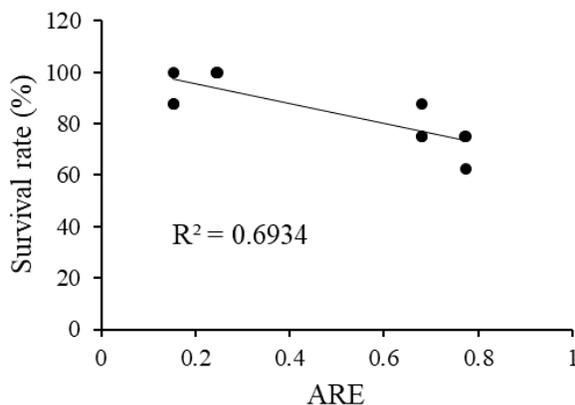


Figure 7: Correlation between RGR (Relative growth rate) (g g⁻¹week⁻¹) and Survival Rate (%)

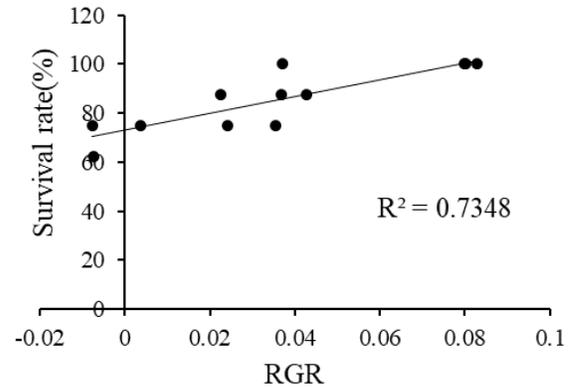


Figure 8: Correlation between different biometric growth indices such as RGR (Relative growth rate) (g g⁻¹week⁻¹), Survival rate (%) and ARE (Apparent recovery efficiency).

Conclusion

Based on the results obtained in the current study, 0.5 gL⁻¹ Albert's solution concentration gave the best growth performance for micro propagated Water Trumpet with a high survival.

Recommendations

The present study focused only on the range of 0.0-2.0 gL⁻¹ of Albert's solution concentration and therefore, further studies are recommended to conduct in the range between 0.0-1.0 gL⁻¹ Albert's solution. It is also recommended to increase the time duration of the experiment to compare the growth performance of plants at the maturation level.

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