

Butterhead lettuce growth under shallow water tables and its recovery on tropical urban ecosystem

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Butterhead lettuce (*Lactuca sativa* var. *capitata*) is a nutrient-rich leafy vegetable beneficial for human health. Lettuce growth and yield performance hampered under water stress conditions. This study aimed to assess its growth and recovery under short-term shallow water conditions in the tropical urban ecosystem. A randomized block design was used with three water table treatments: 16.7 cm, 12.7 cm, and 9.7 cm from the substrate surface. The Results showed that butterhead lettuce is intolerant of excess water, with stunted growth at the 9.7 cm water level, by affecting leaf length, leaf width, leaf initiation, and canopy area. Substrate moisture also indicated excess water at this level. Optimal recovery was observed two weeks after water stress. Leaf length and leaf width were analyzed using zero-intercept linear regression and the results were reliable predictors of leaf area ($y = 0.6076LLxLW$; $R^2 = 0.9694$). In conclusion, butterhead lettuce is sensitive to excess water, as shown by morphological changes, and requires two weeks to recover after water stress.

1. Introduction

The vegetables need of urban communities can be met through the optimization of cultivation in urban areas. Agriculture in urban areas is one of the efforts to support food sustainability (Abdoellah *et al.*, 2023). The benefits of urban farming to the food resilience of urban populations today are beginning to be recognized, especially after the COVID-19 pandemic (O'Hara and Toussaint, 2021; Murdad *et al.*, 2022). In addition, urban farming is an effort to preserve and enhance social space, green infrastructure, and biodiversity (Pradhan *et al.*, 2023). The optimization of urban farming is also essential, especially when reviewed from an aesthetic point of view. More thoroughly, Nicholas *et al.* (2023) stated that urban farming was very beneficial, especially in the environmental, social, and psychological contexts. The benefits of urban farming can also be seen from

the nutritional security and economic perspectives. Lal (2020) reported that beside being beneficial for improving environmental ecosystems, urban farming plays an important role in contributing to food and nutrition security as well as being economically beneficial. In line Ebenso *et al.* (2022) who emphasized that developing urban farming is important in supporting nutritional security. Furthermore, Yuan *et al.* (2022) mentioned that urban farming will increase community income thereby bringing economic benefits on both micro and macro scales.

Climate uncertainty is an issue that must be addressed, especially in tropical ecosystems. According to Sheldon (2019), climate change causes climate uncertainty that impacts ecology and evolution. As a result, this condition requires adaptation for several types of activities, one of which is activities related to agriculture. Climate change has a significant impact on the availability of water on agricultural land. Rainfall with high intensity is an impact of climate change. According to Eccles *et al.* (2019), excess water is one of the impacts of climate change that can occur in the tropics. This condition causes excess water availability, so plants experience excess water stress. In a riparian wet land, similar to this study site, excess water stress can occur through flooding. Several cases of excess water that negatively affect plant growth have been reported, such as tomatoes (Yin *et al.*, 2023) and *Brassica napus* (Guo *et al.*, 2020).

The efforts to find vegetable crops and their cultivation techniques under conditions of excess water stress continue to be developed. Susilawati and Lakitan (2019) reported that chickpea (*Phaseolus vulgaris* L.) plants were able to grow at a water table of 20 cm below the soil surface. Meanwhile, in other plants, such as tomato plants, 5 cm and 10 cm below media surface, did not reduce leaf growth rate, specific leaf weight, and leaf water content (Meihana *et al.*, 2017). Recovery is an effort to restore plant growth performance after experiencing excess water stress. Hud *et al.* (2023) stated that the recovery ability of white cabbage was considered satisfactory after experiencing excess water stress. However, each plant has its period to recover from excess water stress. Nazari *et al.* (2019) emphasized that the longer the recovery period, the better the changes after experiencing the effects of hypoxia, especially in the roots. Meanwhile, some plants show a better response after recovering from excess water stress, as has been reported in grass pea (Wiraguna *et al.*,

2021).

Experiments on the effect of a water table on vegetable growth, particularly on butterhead lettuce, have been few and far between. This validates the fact that vegetables are a kind of plant that is susceptible to stress. A shallow water table experiment on butterhead lettuce will provide an understanding, particularly of this lettuce's level of tolerance to climate uncertainty, particularly in excess water conditions. The study was aimed to evaluate the growth of butterhead lettuce on several shallow water tables as well as its ability to recover afterward.

2. Materials and Methods

Research site and agroclimatic conditions

The research was carried out at the Jakabaring Research Facility in Palembang, South Sumatra, Indonesia (104°46'44''E and 3°01'35''S). The study began on July 18, 2023, and ended on September 16, 2023. The study site is a lowland urban area with a tropical ecosystem. The study area has entered the dry season, which is characterized by low rainfall, but air humidity is high, often exceeding 70% (Fig. 1).

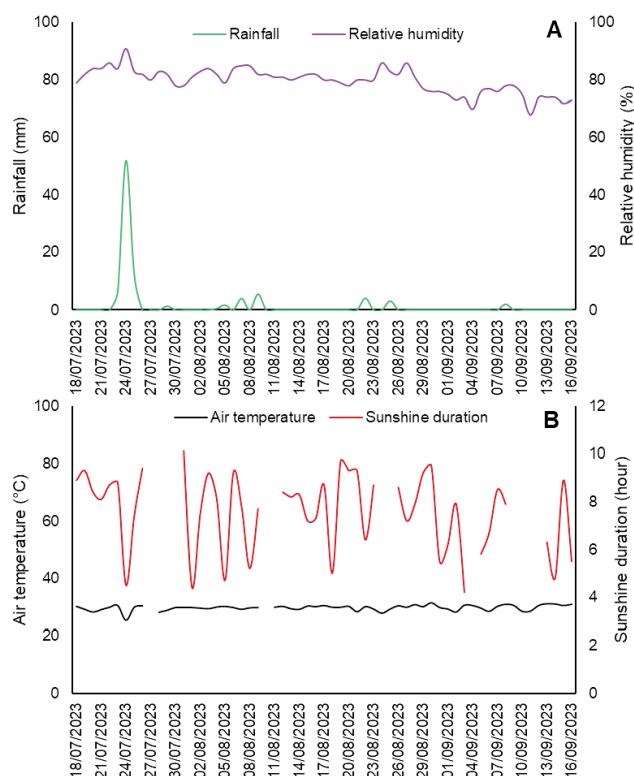


Fig. 1 - Daily rainfall-relative humidity (A) and air temperature-sunshine duration (B) at the research location during the research was carried out. Source: Meteorological, Climatological, and Geophysical Agency, 2023.

Research protocol

Twenty days old butterhead lettuce seedlings were used in the study. The seedlings were transplanted into pots (27.5 cm of height and diameter). As the growing substrate, the pots were filled with topsoil. The plants that had been transplanted to the growing substrate were placed in an open field until 4 weeks after transplanting. Fertilization was performed by NPK (16:16:16) fertilizer after 3 weeks, and watering was performed regularly in the afternoon when it was not raining.

In 4 weeks after transplanting, butterhead lettuce was treated with water maintaining 16.7 cm water tables (WT1), 12.7 cm (WT2), and 9.7 cm (WT3) from the substrate surface (Fig. 2). Each treatment was repeated 3 times. This stage was carried out in an experimental pond measuring 4 m (length) x 2 m (width) x 0.5 m (height). The pond was equipped with an outlet to allow water to flow out in the event of excessive rain. As a result, water level can be controlled based on the water table treatments. During this stage, the plants get their water from the bottom of the pot via capillary water movement, so no watering is required.

After 7 days of water treatments (WT1, WT2 and WT3) butterhead lettuce was return to the open area. As additional treatments, several recovery times were treated, including no recovery, one week of recovery, two weeks of recovery, and three weeks of recovery. During this phase, butterhead lettuce was watered minimally and only when there was no rain for three days in consecutive days.

Data collection

Butterhead lettuce growth data was collected consisting of individual leaf growth, canopy diameter,

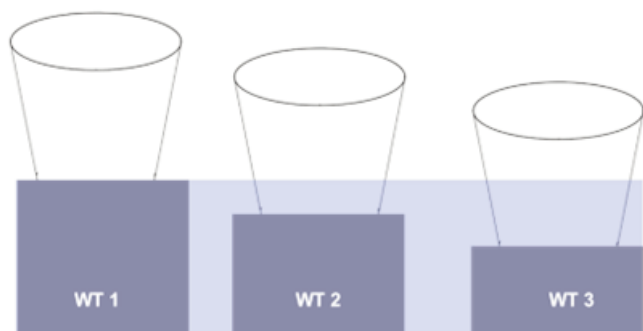


Fig. 2 - The illustration of the shallow water table treatments application. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

canopy area, fresh weight and dry weight organ. Individual leaf growth was monitored daily for length and width, starting when the leaf was fully unfolding. The canopy diameter was measured daily on the cross-sectional widest canopy to track canopy diameter growth. The butterhead lettuce canopy area and leaf number were measured weekly. The canopy area was measured using the image scanner Easy Leaf Area software for Android (Easlon and Bloom, 2014). Meanwhile, substrate moisture was measured using a moisture meter (Lutron Soil Moisture Meter PMS-714).

Destructive observation was conducted to collect fresh weight and dry weight data of plant organs. To obtain dry weight, each plant organ was thinned and then dried in an oven at 100°C for 24 hours.

Experimental design and statistical analysis

The study used a randomized block design. The shallow water table treatments consisted of 16.7 cm (WT1), 12.7 cm (WT2), and 9.7 cm (WT3) from the substrate surface. All data collected were subjected to analysis of variance (ANOVA), then significance among treatments using the least significant difference (LSD) at $P < 0.05$. The significance of differences among treatments was also tested using independent t-test at $P < 0.05$. The analysis was performed using RStudio (v2023.06.0+421) for Windows 10 (Rstudio team, PBC, Boston, MA, USA). Meanwhile, data trend on the selected variables were analyzed using Microsoft Excel for Windows 10 (Microsoft Inc., Redmond, Washington, USA).

3. Results

Individual leaf growth

Butterhead lettuce leaf length increased up to 5 days after leaf unfolding (DAU). Furthermore, beginning at 8 DAU, leaf length gradually stagnated. The shallowest water table (WT3) affected the inhibited leaf length during the treatment (Fig. 3). Leaf widening was also observed with the shallow water table treatment. Butterhead lettuce grown in WT1 produced larger leaves. Meanwhile, butterheads planted at the shallowest water table (WT3) experienced inhibited leaf widening, resulting in narrower leaves. The butterhead lettuce leaves, on the other hand, continued to widen until 9 DAU. As a result, the width of the leaves stagnated and experienced senescence, which caused the tips of the

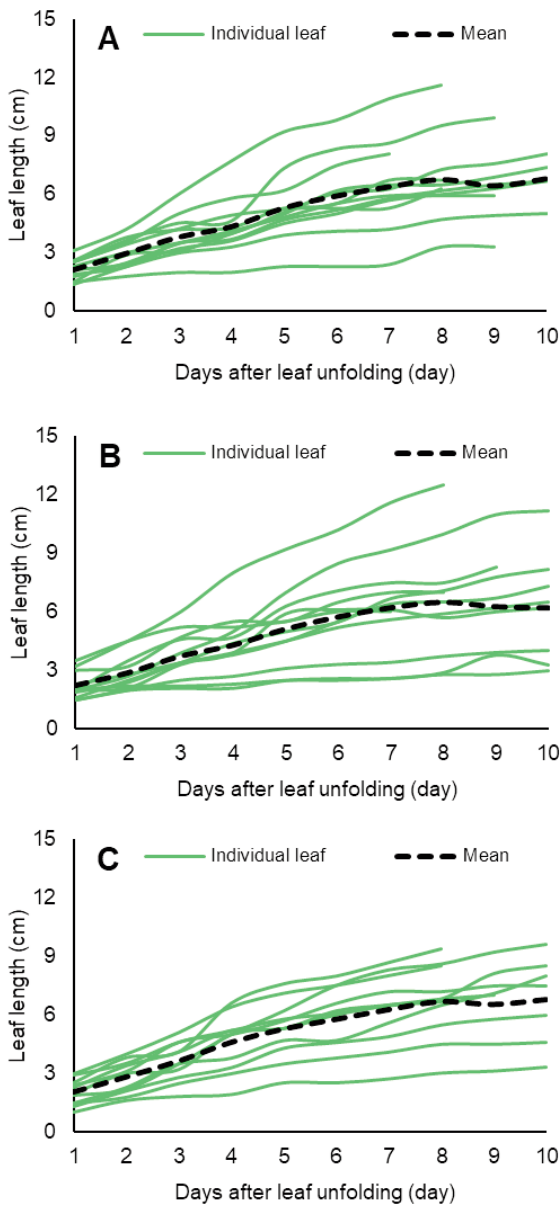


Fig. 3 - Daily leaf length of butterhead lettuce on different shallow water tables. The shallow water tables (WT) consisted of WT1 (A), WT2 (B), and WT3 (C). The measurement was carried out when leaf was fully unfolded. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

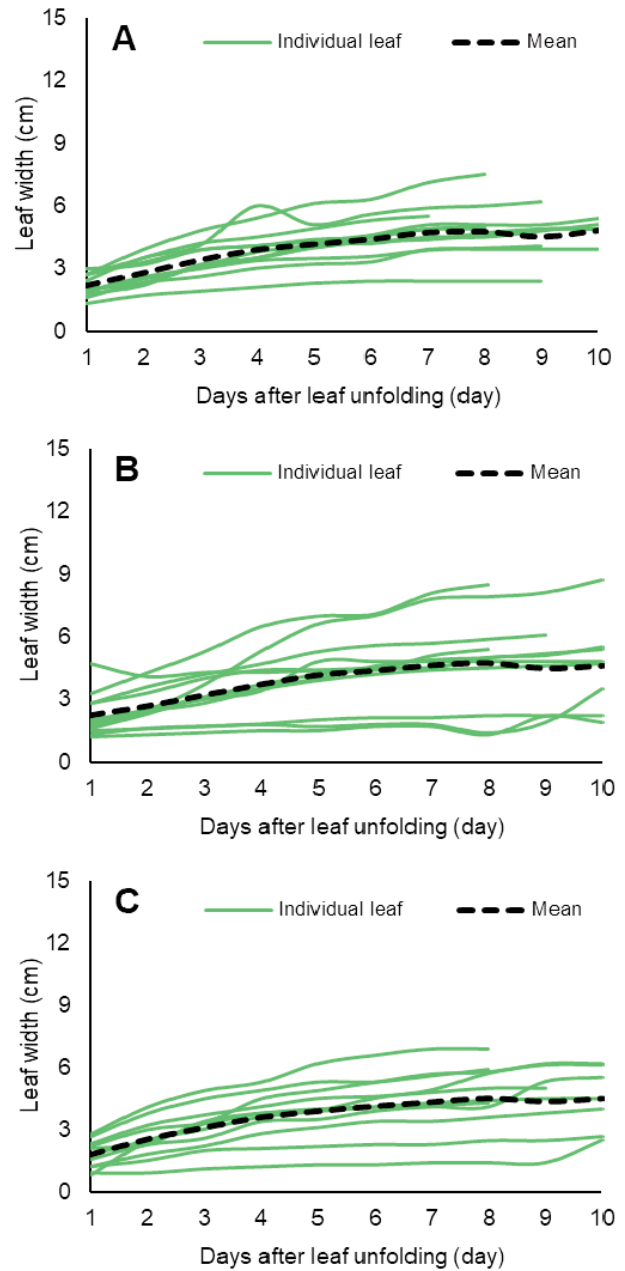


Fig. 4 - Daily leaf width of butterhead lettuce on different shallow water tables. The shallow water tables (WT) consisted of WT1 (A), WT2 (B), and WT3 (C). The measurement was carried out when leaf was fully unfolded. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

leaves to dry out, resulting in a decrease in leaf width (Fig. 4).

The leaf shape is represented by the leaf length-width ratio. Leaves with a length-width ratio over one indicate elongated leaf growth. If the leaf length-width ratio is less than one, it indicates the leaf growth has widened. There was no difference in the

dynamics of changes in the shape of butterhead lettuce leaves in each water table treatment. Butterhead lettuce leaves widened as they aged as a whole (Fig. 5).

Different water table treatments influenced the growth canopy of butterhead lettuce. Butterhead lettuce with the shallowest water table (WT3)

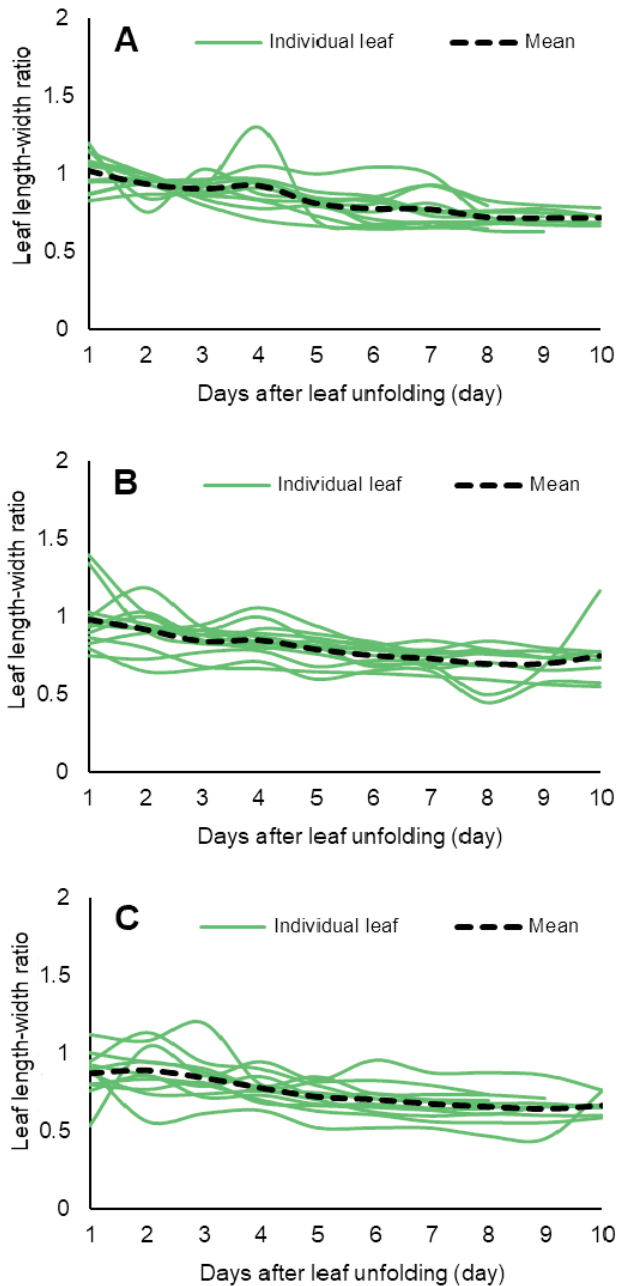


Fig. 5 - Daily leaf length-width ratio of butterhead lettuce on different shallow water tables. The shallow water tables (WT) consisted of WT1 (A), WT2 (B), and WT3 (C). The measurement was carried out when leaf was fully unfolded. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

exhibited lower canopy growth. The stunted growth of leaves in WT3 resulted in a low canopy width. In contrast, better leaf growth in WT1 and WT2, respectively, resulted in a wider canopy (Fig. 6).

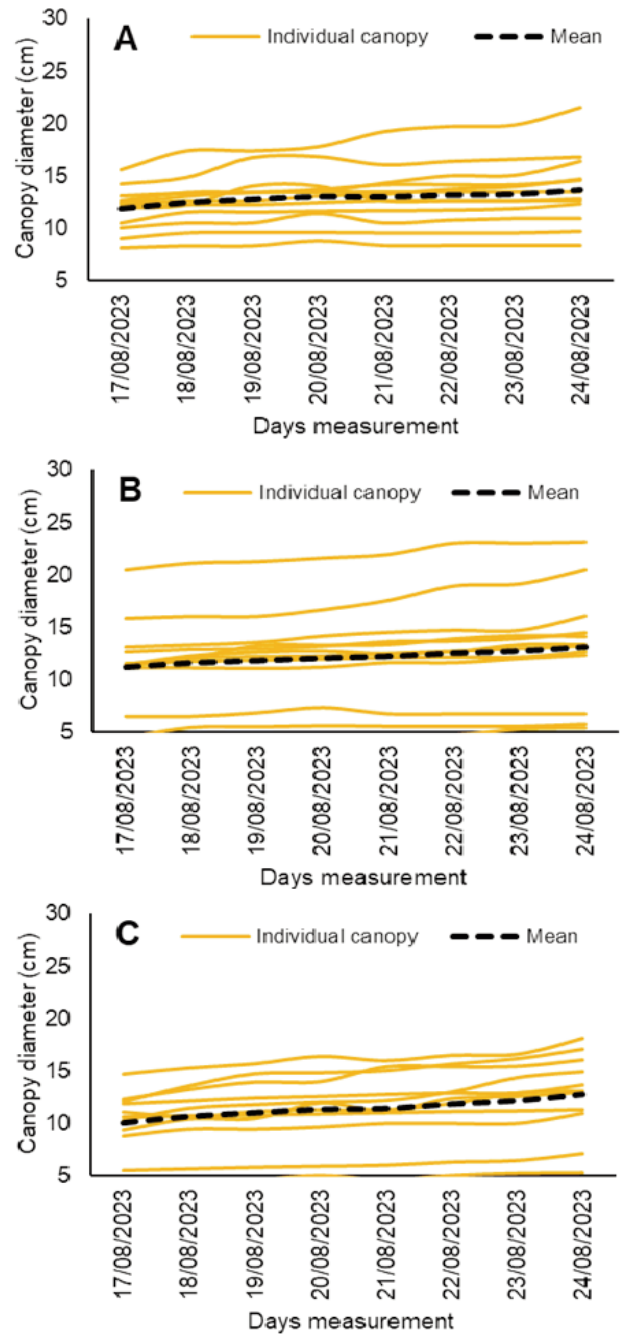


Fig. 6 - Daily canopy diameter of butterhead lettuce on different shallow water tables. The shallow water table consist of WT1 (A), WT2 (B), and WT3 (C). WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

Weekly growth of butterhead on different shallow water tables

The WT1 exhibited a better trend for butterhead lettuce leaf initiation than the WT2 and WT3. However, statistically, no significant difference was found between the three treatments (WT1, WT2, and

WT3). The leaf number grows in line with the plant's age. The increase in leaf number follows a polynomial curve (Fig. 7).

The canopy area of butterhead lettuce differed among the treatments. During early vegetative growth (1 and 2 WAT), WT1 exhibited the most expansive canopy area, indicating significant differences in the canopy area. The canopy of butterhead lettuce showed no difference at later ages. However, when compared to the WT2 and WT3 treatments, the trend of canopy area growth of butterhead lettuce in WT1 remained higher, with canopy area growth following a polynomial curve.

There are signs that WT1's canopy area growth has stagnated, especially after 4 WAT, when the WT1 butterhead lettuce canopy area is almost the same as the WT2 treatment (Fig. 8).

Butterhead growth performance during recovery time

After recovery from water stress, the production of edible and non-edible butterhead lettuce leaves fluctuated. All treatments showed peak edible leaf production at 2 weeks after recovery (WAR). At this time, butterhead lettuce in WT3 has the highest edible leaf production. Meanwhile, non-edible leaf production was highest in WT2 compared to the

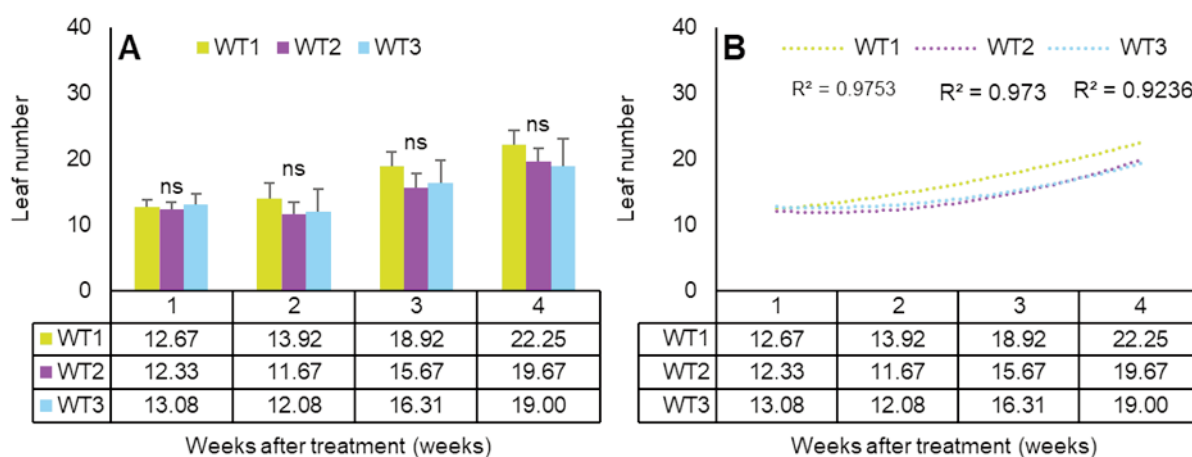


Fig. 7 - Leaf number of butterhead lettuce (A) and their trend (B) during on different shallow water table. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. The ns indicated each treatment non-significant at LSD0.05.

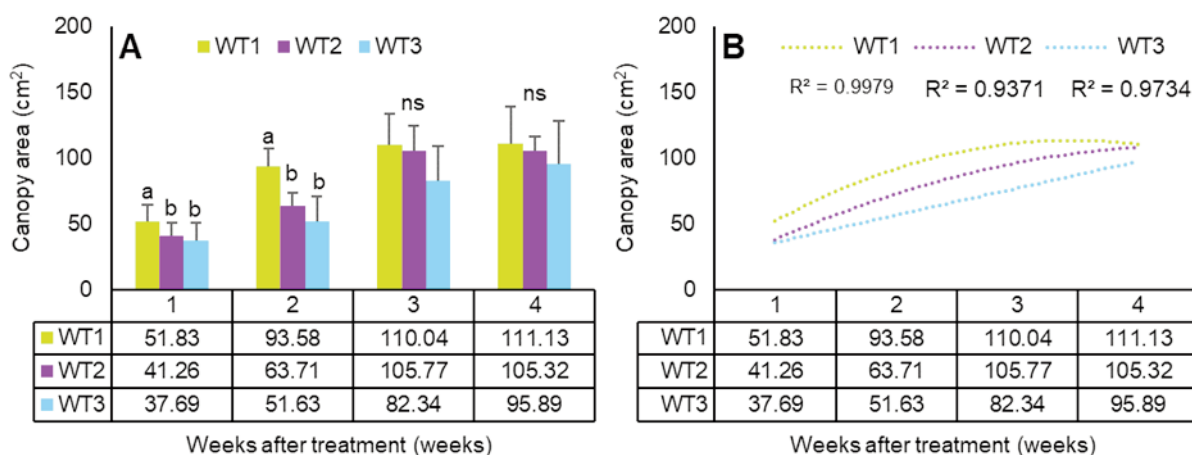


Fig. 8 - Canopy area of butterhead lettuce (A) and their trends (B) on different shallow water table. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. The different letters on bar indicated each treatment significant different at LSD0.05. The ns indicated each treatment non-significant at LSD0.05.

other water table treatments (WT1 and WT3) (Fig. 9). Additionally, according to shoot fresh weight, butterhead lettuce shoots on all shallow water tables reached their peak growth at 2 weeks after recovery (WAR). Following the recovery time, the WT3 treatment showed improved shoot growth in

comparison to WT 1 and WT 2 (Fig. 10).

Leaf estimation

The butterhead lettuce leaf has a morphology with pinnate veins. The pinnate leaf blade makes it possible to assign leaf length (LL) and leaf width (LW)

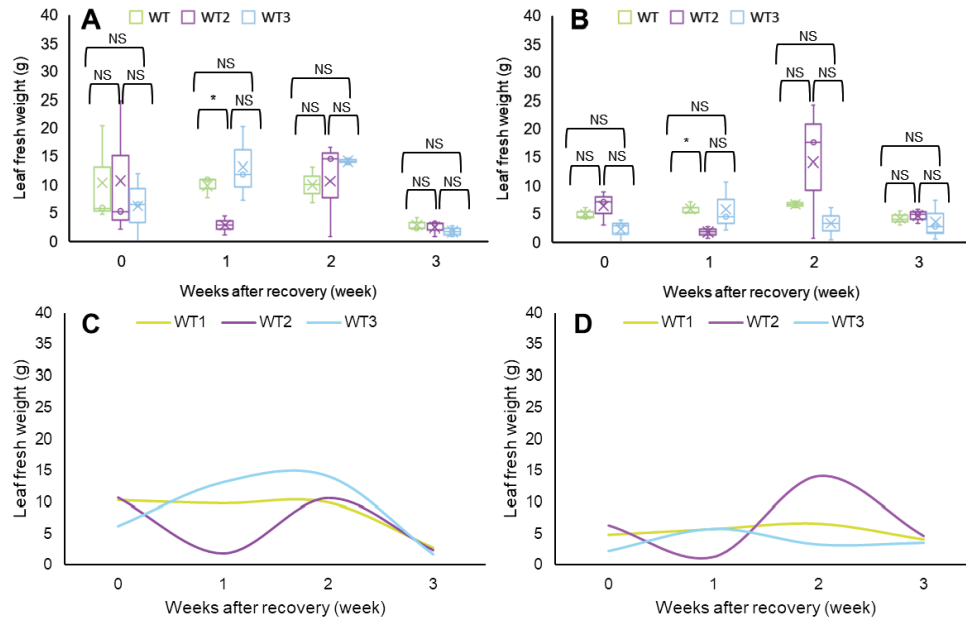


Fig. 9 - Edible leaf and non-edible leaf of butterhead lettuce (A-B) and their trends (C-D) on recovery from different water tables. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. NS: non-significance different based on independent t-test at $P < 0.05$; *: significance different based on independent t-test at $P < 0.05$.

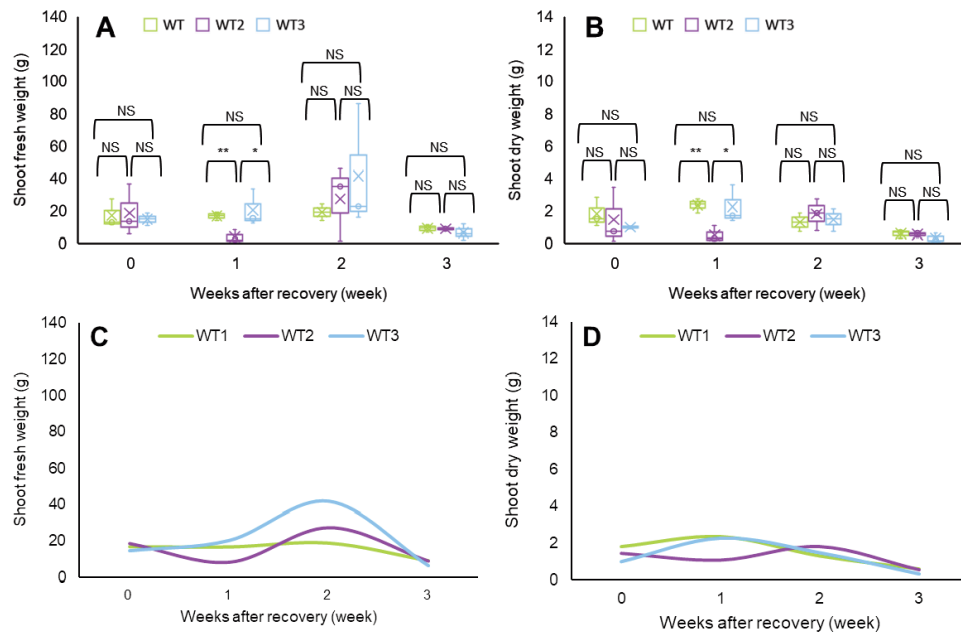


Fig. 10 - Fresh weight and dry weight of butterhead lettuce shoot (A-B) and their trends (C-D) on recovery from shallow water table. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. NS: non-significance different based on independent t-test at $P < 0.05$; *: significance different based on independent t-test at $P < 0.05$; **: significance different based on independent t-test at $P < 0.01$.

as primary predictors. The results showed that the combination of LL x LW using the zero-intercept linear regression type most reliably represented leaf area ($R^2 = 0.9694$) (Table 1).

The physiological capacity of a leaf is determined by its leaf area. The findings revealed that increased leaf fresh weight was linearly related to increased leaf area ($R^2=0.8744$). This suggests that larger leaves have more biomass and water. The opposite condition occurred in narrow leaves (Fig. 11).

Water status on different water table treatment

Butterhead lettuce grown in the WT3 treatment receives more water than those grown in WT1 and WT2. This is an indication of excess water, as indicated by the moisture level of the substrate in each treatment. The shallow water table in WT3 causes water to fill the substrate pores faster, resulting in higher substrate moisture than in the other treatments. As a result of this condition, the aerobic space in the WT3 substrate is lower than in the WT1 and WT2 substrates (Fig. 12).

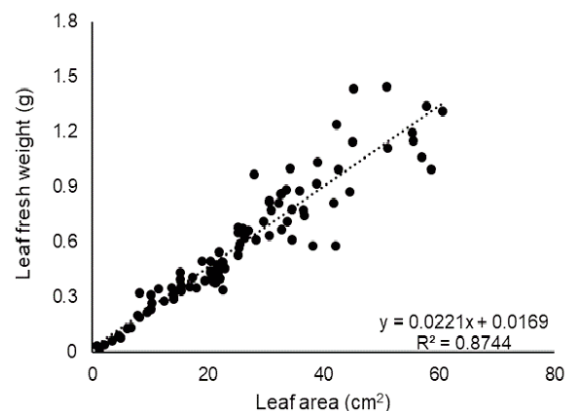


Fig. 11 - Relation between leaf area and leaf fresh weight of butterhead lettuce.

4. Discussion and Conclusions

Plant growth response under excess water conditions

The shallower the water table, the deeper the pot is submerged, hence the less aerobic space available to the plant. Aerobic space is incredibly beneficial to

Table 1 - Butterhead leaf estimation involve leaf length (LL), leaf width (LW), and LL x LW as predictors

Predictors	Regression type	Equation	R ²
Leaf length (LL)	Linear	Y= 5.0467(LL)-12.66	0.8674
	Exponential	Y= 2.6911e ^{0.2656(LL)}	0.7598
	Logarithmic	Y= 29.963ln(LL)-32.71	0.7534
	Quadratic	Y= 0.1327(LL) ² +3.0079(LL)-5.7844	0.8752
	Power	Y= 0.6121(LL) ^{1.7939}	0.8681
	Zero intercept linear	Y= 3.5771(LL)	0.9491
	Zero intercept quadratic	Y= 0.2172(LL) ² +1.5467(LL)	0.8715
Leaf width (LW)	Linear	Y= 9.0909(LW)-18.948	0.8464
	Exponential	Y= 1.8915e ^{0.4829(LW)}	0.6645
	Logarithmic	Y= 38.424ln(LW)-33.461	0.7777
	Quadratic	Y= 0.118(LW) ² +7.908(LW)-16.239	0.8469
	Power	Y= 0.6148(LW) ^{2.2685}	0.8055
	Zero intercept linear	Y= 5.5761(LW)	0.9312
	Zero intercept quadratic	Y= 0.7056(LW) ² +1.508(LW)	0.8310
LLxLW	Linear	Y= 0.5548(LLxLW)+2.9686	0.8813
	Exponential	Y= 6.8069e ^{0.0267(LLxLW)}	0.6010
	Logarithmic	Y= 17.35ln((LLxLW)-34.847	0.7874
	Quadratic	Y= -0.0027(LLxLW) ² +0.8335(LLxLW)-2.3609	0.9039
	Power	Y= 0.5508(LLxLW) ^{1.0323}	0.8780
	Zero intercept linear	Y= 0.6076(LLxLW)	0.9694
	Zero intercept quadratic	Y= -0.002(LLxLW) ² +0.7422(LLxLW)	0.9026

Coefficient of determination (R²) indicated strength level of each predictor and regression

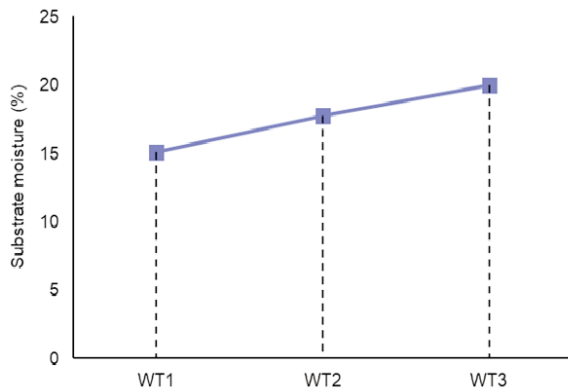


Fig. 12 - Substrate's water status on different shallow water table as indicated by substrate. moisture. The shallow water table consist of WT1, WT2, and WT3. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. The measurement was conduct at 4 weeks after treatment.

plants as a source of oxygen for many kinds of crucial metabolic activities. Oxygen plays an essential role in several metabolisms in plants, including respiration, carbohydrate formation, protein synthesis, and nutrient solubility (Moreno Roblero *et al.*, 2020; Xu *et al.*, 2020). Oxygen plays an important role in the growth of some soil microorganisms (Wichern *et al.*, 2020). As a result, if the amount of oxygen in the pot is insufficient, plant growth will decrease.

Based on the results, butterhead lettuce grown at the shallowest water table (WT3) showed a stunted growth response. Actually, each plant has different tolerance abilities in excess water conditions. As a consequence of excessive water stress, each plant exhibits specific symptoms. In the case of butterhead lettuce, the plants exhibited stunted leaf and canopy growth. On other hand, plant growth performance was inhibited, as evidenced by data trend of fresh weight and dry weight of edible leaf and shoot on 0 week after recovery before recovery (WAR) (Fig. 9 and 10). Plants respond to excessive water stress by changing their physiology, anatomy, and morphology (Jia *et al.*, 2021; Kumar *et al.*, 2022). According to Zhou *et al.* (2020), plants grown under excess water, change metabolic energy, respiration, photosynthesis, and endogenous hormone regulation.

Hypoxia conditions further hinder plant growth. Some leafy vegetables, such as tomatoes (Tareq *et al.*, 2020) and broccoli (Casierra-Posada and Peña-Olmos, 2022), have been shown to have stunted

growth. However, some plants, such as white cabbage, are potentially water-tolerant (Hud *et al.*, 2023). Thus, excess water is a problem for some crops, including butterhead lettuce. In response to excess water, several approaches have been tried, including enriching CO₂ in the substrate (Pérez-Romero *et al.*, 2019) and utilizing the role of ethylene (Khan *et al.*, 2020).

Recovery as an effort to restore plant growth performance

Recovery by returning to open areas was aimed to restore the butterhead lettuce growth after experiencing excess stress. Plant organ architecture and physiological regulation will improve as a result of recovery (Yin and Bauerle, 2017). Depending on the level of stress, each plant requires a different amount of time to recover and return to average or near-normal growth. Our observations indicate that butterhead lettuce takes 2 WAR to restore its growth performance after being grown in shallow water table treatments, was shown clearly in the WT3. The fresh weight of edible leaf and plant organs indicates this. In another case, Nazari *et al.* (2019) found that even 4 days after recovery, hypoxia did not affect *Cicer arietinum*.

After recovery, each plant treated with a different shallow water tables demonstrated a different level of endurance. Interestingly, the shallowest water table (WT3), which had stunted growth when treated with shallow water, had the best recovery growth. Because of the residual pretreatment, the water availability in WT3 was adequate, resulting in better growth. WT3, the shallowest water table, causes the most water retention in the substrate when compared to WT1 and WT2. Plants use excess water during the recovery process since they are rarely watered during this period. According to Bateman *et al.* (2019), substrates with adequate water storage will promote plant growth.

Leaf area estimation and leaf morphological characterization

The role of the length and width of butterhead lettuce leaf as a predictor is essential for a plant with a pinnate leaf shape. These predictors were also tested on leaves with similar leaf shapes, such as citrus (Muda *et al.*, 2023) and Swiss chard (Ria *et al.*, 2023). Furthermore, complex leaf shapes, such as *Amorphaphalus mullieri*, can be estimated by considering leaf morphology (Nurshanti *et al.*, 2022).

Furthermore, the choice of regression type influences predictor reliability in predicting leaf area. According to the findings, the zero-intercept linear regression with the LL x LW predictor was the most dependable. The logic behind zero intercept regression is that if the predictor is 0, the leaf area will also be 0 (Lakitan *et al.*, 2022). The use of zero intercept regression in estimating leaf area has been confirmed in cassava (Lakitan *et al.*, 2023) and chaya (Gustiar *et al.*, 2023).

Butterhead lettuce has been proven to be intolerant of excess water in growing environments, such as at a water table of 9.7 cm from the substrate surface. Butterhead lettuce that has experienced excess water stress needs recovery with the most optimal recovery time within 2 weeks. Butterhead lettuce has a pinnate leaf morphology, and leaf area can be estimated using the formula $y = 0.6076 \text{ leaf length} \times \text{leaf width}$.

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