JJBS

Jordan Journal of Biological Sciences

Volume 16, Number 1,March 2023
ISSN 1995-6673
Pages 157 – 164
https://doi.org/10.54319/jjbs/160119

Leaf Blade Growth and Development in Red, Pink, and Yellow Petiole Cultivars of the Swiss Chards Grown in Floating Culture System

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Received: March 28, 2022; Revised: June 25, 2022; Accepted: July 6, 2022

Abstract

Adaptability of the Swiss chard to the tropical climate opens opportunity to grow this leafy vegetable all year around. Colorful petiole and leaf blade of this vegetable have attracted urban community to grow them. Objectives of this research were to study the morphological and growth characteristics of the Swiss chard, especially leaf growth rate, time to reach full size, and non-destructive measurement of the leaf area. The Swiss chards used were red, pink, and yellow petiole cultivars. The plants were cultivated using the floating culture system. Relative leaf elongation and widening rates were higher at early leaf development, i.e. soon after the leaf blade was unfolded. Then, the rates were gradually declined and completely halted at about 12 days. In this study, combination of the zero-intercept linear model and the length × width (LW) as predictor were accurate for estimation leaf area (LA) of the red (R^2=0.981), pink (R^2=0.975), and yellow (R^2=0.980) petiole cultivars. The non-destructive measurement on selected morphological and/or weight traits should be useful for continuously monitoring of leaf growth, predicting yield at any time during plant growth, and determining the time to harvest.

Keywords: continuous monitoring, time to harvest, non-destructive measurement, yield prediction, zero-intercept regression.

1. Introduction

The western part of the Indonesian archipelago is classified as the tropical rain forest climate zone and characterized by high annual rainfall. Waterlogging occurs periodically in lowlands and coastal areas during rainy season. Floating culture is a reliable solution for avoiding the vegetables from submergence during the rainy season (Jaya et al., 2021; Siaga et al., 2018).

Swiss chard is a suitable leafy vegetable for cultivation during cool summer in the temperate climate zone, yet this vegetable can also tolerate the heat and humid tropical climate as long as water is available for maintaining soil moisture. Swiss chard is also grown well under full sunlight or partial shade. In the tropics, this vegetable is potentially grown all-year around. Leaf is obviously the most valuable organ in leafy vegetable.

Leaf has some specific roles, including capturing sunlight, absorbing CO_2, controlling inner temperature, and synthesizing carbohydrate. Leaf is also a good source of vitamins, minerals, fibers, pigments, antioxidants, and other beneficial substances for human health. Leaves also very significantly contribute to quality of environment. Furthermore, vibrant colors of leaf blade and petiole, such as in Swiss chard plant, also contribute to aesthetic value in urban living space.

Studies have focused on leaf from many different perspectives has aroused (Katifori, 2018; Smithers *et al*., 2019). Vanhaeren *et al*. (2015) explained that leaf emerged as a group of cells at the shoot apical meristem, then developed into planar, a complicated organ through different interrelated cellular events. Leaf development is driven by cell proliferation and cell expansion. However, active suppression on leaf meristem decelerated and, at the end, halted leaf growth (Alvarez *et al*., 2016)

Leaf shape and size were controlled by multiple genes. Modern technology helped to identify numerous genes that contributed to the final size of leaves and unraveled the complex cellular and molecular mechanisms that underlie leaf growth. Numerous genes have been identified to play significant role in inducing cell proliferation and/or size during leaf growth (Vercruysse *et al*., 2020).

The problem in the cultivation of leafy vegetables, such as Swiss chard, is the difficulty in determining the right time for harvesting in order to get an optimal quantity of yield and excellent quality of vegetables, namely the leaves are not too old at time of harvest. It was hypothesized that the Swiss chard leaf enlargement follows the Sigmoid curve and takes less than 10 days to reach the optimal leaf size.

Objectives of this research were to compare amongst Swiss chard with different petiole colors on their leaf morphological and growth characteristics, including probable changes in two-directional leaf growth, patterns

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of midrib elongation and blade widening in growing leaf, number of days for individual leaf to reach its full size, development of accurate and reliable model for non-destructive leaf area estimation, and monitoring daily growth of Swiss chard leaves.

2. Materials and Methods

The research was conducted during dry season (31^o C and RH ± 51%) in tropical lowland climate at the Jakabaring Outdoor Research Facilities, Palembang, Indonesia. Planting material used was seeds of three Swiss chard cultivars with different petiole colors, i.e. red, pink, and yellow (Figure 1). The seeds were soaked in warm water (30^oC) for 15 minutes for enhancing imbibition process, then sown in seedling trays filled soil-chicken manure mixture (2:1 v/v).

The seedlings with two true leaves were transplanted to plastic pot at 14 days after sowing (DAS). The size of pot was 27.5 cm in inner-upper diameter and 19.8 cm in inner-lower diameter. The pots were loaded with the soil-manure mix similar to those used in nursery up to 20 cm in height. Prior to transplanting, the mix was pre-treated with bio-sterilant at dose of 2 gram/liter. Each pot was applied with 200 ml bio-sterilant solution. Nutrient was applied at non-flooded, well-aerated, upper layer of the substrate within each pot.

A floating cultivation system was conducted in an experimental pond. Three cultivation rafts were used. The raft dimensions were 2.0 m (length) × 1.0 m (width) × 0.078 m (height); constructed using 66 units of 1500 mL polyethylene terephthalate (PET) bottles as the floater. The water-substrate interface (WSI) was adjusted to 1.5 cm depth. The WSI is the thin (1-2 cm) water-saturated layer at bottom part of growing substrate which maintaining physical connection between the substrate and water surface. The pots were placed on the raft. The raft was submerged in water such that 1-2 cm growing substrate within the pots were always in contact with water surface. Each pot has 4 holes at the bottom. Substrate moisture is relatively stable between 15% to 20% due to the upward movement of water through the bottom 4 holes of the pot driven by the capillarity force.

Split plot experimental design was used. Three cultivation systems were assigned as the main plot, i.e., floating bottom-wet, and conventional. On each raft in floating culture consisted of 3 cultivars of Swiss chards were assigned as the subplots, 3 replications in each cultivar, and 2 pots in each replication. In total, 18 pots were placed on each raft in floating culture system.

2.1. Data collection

Length of leaf midrib (L) and width of leaf blade (W) were daily measured for 16 consecutive days and used as predictors for non-destructive estimation of LA. Measurement begins when the leaves begin to be unfolded on each individual leaf.

Leaf blade discontinued to enlarge in less than 2 weeks. Multiplication of the L×W was used as a secondary predictor for the LA. Accuracy of the LA estimation model was based on value of the coefficient of determination (R^2). The developed model was validated by comparing estimated value of LA and direct measurement of LA using a digital image analysis software LIA32, designed by Kazukiyo Yamamoto, Nagoya University, Japan.

Figure 1. Swiss chard with red, pink, and yellow petioles

2.2. Data analysis

Various types of regression models and predictors had been evaluated, and it was found that there were 3 most accurate models for leaf area estimation, namely: (a) the zero-intercept linear model uses the length × width of the leaf blade as a predictor; (b) the power and (c) the polynomial models if the length or width of the leaf blade is used as a predictor (Lakitan *et al*., 2017; 2018a; 2021). Therefore, in this study, the LA estimation was conducted using the zero-intercept linear, quadratic, and power regressions. The three regressions were intentionally chosen based on their performances in the previous studies.

Predictors used were L, W, and L×W. Curves of the relative leaf elongation, the relative leaf widening, and the leaf area enlargement were fitted using polynomial order-3 regression for mimicking the S-curve or reversed S-curve. Other secondary parameters were also calculated, including absolute leaf elongation rate (LER), absolute leaf widening rate (LWR), relative LER, relative LWR, length/width (L/W) ratio, average days to full leaf size (DFS), and non-destructive LA estimation. Calculations of the growth analysis formula were based on Hunt (2012).

3. Results

Leaf blade growth is three directional, i.e. length, width, and thickness; however, leaf thickened did not significantly contribute to light capturing activity, CO2 uptake, transpiration, and heat exchange. Thickness does not contribute significantly (<1%) to the total surface area of the leaves, except for succulent plants. The stomata are also never on the thickness edge of the leaf blade. Instead, the main leaf area is determined by length and width of the blade. Therefore, the two directional growth of midrib elongation and blade widening are the focus in this study (Figure 2). Midrib elongation rate of the yellow-petiole chard was significantly faster (7.69 mm/d) than those of red- and pink-petiole chard. The elongation rates of red- and pink-petiole chard were slightly more than 6 mm/d. The blade widening rates in all three cultivars of Swiss chard were almost similar at around 3.3 mm/d. Higher daily midrib elongation rate in yellow-petiole chard morphologically shaped its leaf to a slimer leaf blade compared to the other two cultivars of Swiss chard.

Ratio of length/width reflects shape of the leaf. The higher ratio value indicates a slimer leaf shape. Most of leaves have ratio of length/width higher than 1. Leaf can change its shape through a gradual process. In Swiss chard leaf, the ratios were 3.26 (red), 3.27 (pink), and 3.66 (yellow) at the early half of their development, then decreased to 2.08, 2.07, and 2.53, respectively, at the rest half, until the leaves stopped expanding. The leaves transformed into a more rounded shape as they got older. Midrib elongation and blade widening rates of individual leaf in all three cultivars of Swiss chards were relatively consistent (Figure 3).

Days required to reach full size leaf were significantly different between Swiss chard plants with red and yellow petiole colors. The fastest relative elongation and widening rates occurred at the first day after the leaf blade unfolded (Table 1). This phenomenon was mainly associated with rapid cell multiplication in the young leaves. Midrib length on the first measurement was less than 5 mm.

Relative leaf elongation and widening rates were very high at early growth in all Swiss chard cultivars studied but then gradually declined and completely halted at age less than 2 weeks (Figure 4). Knowledge on when the leaf blade stops to expand is beneficial for vegetable farmers, since this knowledge can be used as base of decision on the best time to harvest the specified leaf. Furthermore, farmers can develop their schedule for leaf harvesting of Swiss chard plants if the frequency of new leaf developed has also been known.

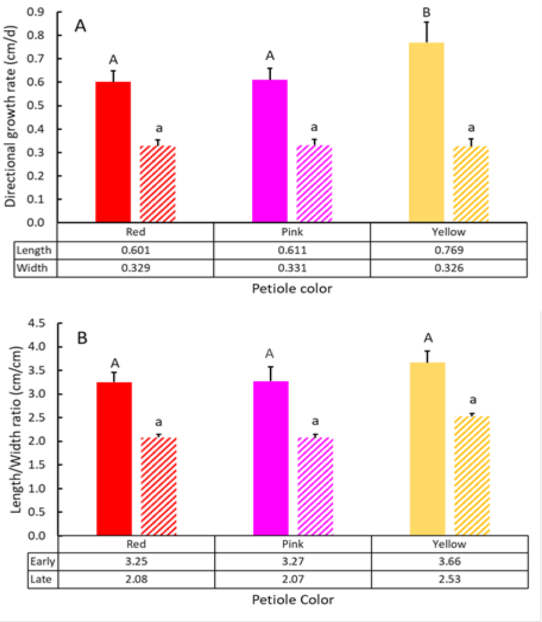

Figure 2. The two-directional growth of the leaf and leaf/width ratio in red-, pink-, and yellow-petiole Swiss chards. Solid bar is for leaf length and striped bar is for leaf width. Means followed by different letter was significantly different at $LSD_{0.05}$. The data in the table is used to show the magnitude of the difference.

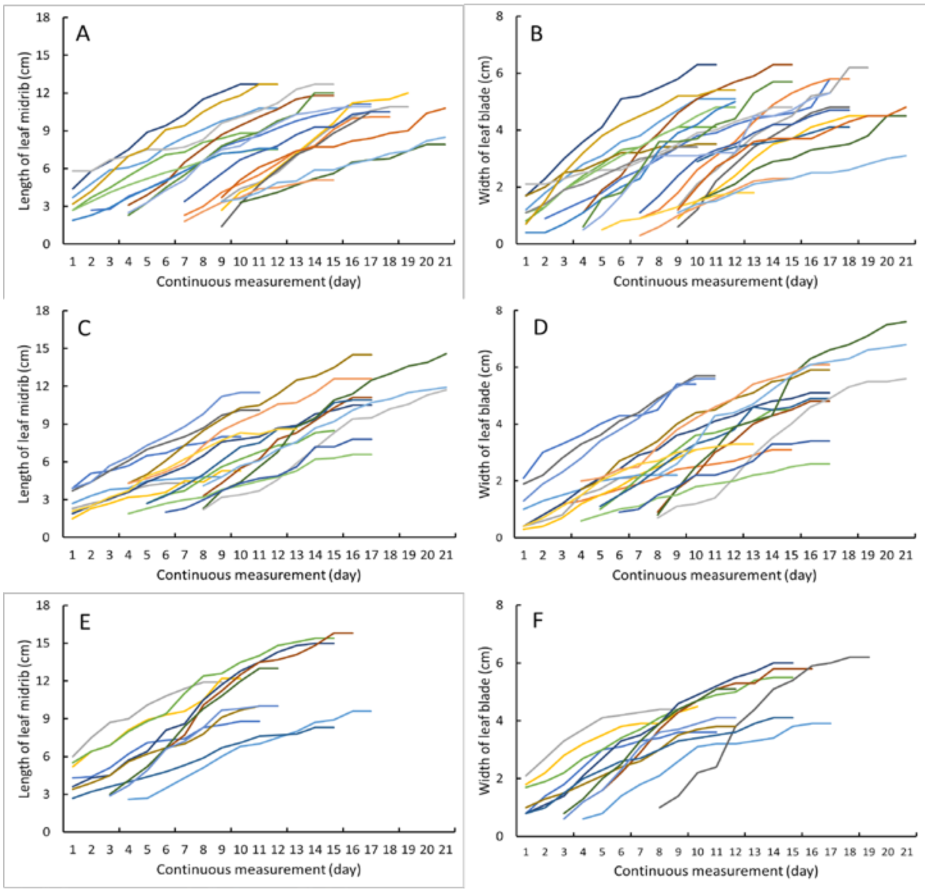

Figure 3. Elongation of midrib (A, C, and E) and widening of blade (B, D, and F) of individual leaf in Swiss chard with red (A-B), pink (C-D) and yellow (E-F) petiole colors. Each line represents the monitored individual leaf.

Table 1. Some leaf growth characteristics in red-, pink-, and yellow-petiole Swiss chard

	Growth characteristics	Petiole color		
		Red	Pink	Yellow
1.	Average number of days to reach full size leaf (d) [a]	11.60 ± 0.36 a	12.26 ± 0.56 b	12.00 ± 0.59 ab
2.	Fastest relative elongation rate (mm/mm/d) [b]	2.697	2.358	2.045
3.	Fastest relative widening rate (mm/mm/d) [b]	5.514	4.786	4.116

[a] Count was started at the first day since leaf blade unfolded. Means followed by different letter was significantly different at $LSD_{0.05}$.

[b] Predicted value using polynomial order-3 regression at the $R^2 > 0.95$ for both elongation and widening.

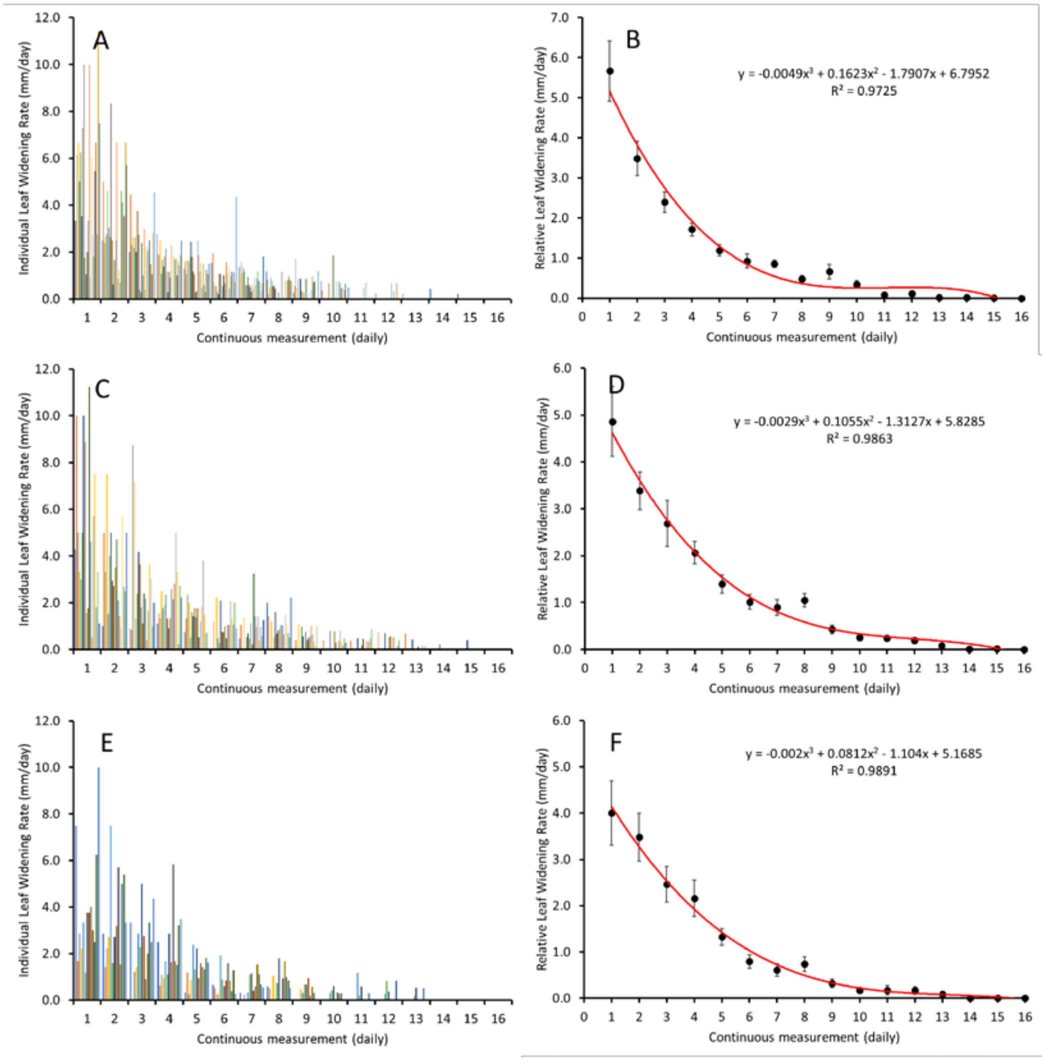

Figure 4. Elongation rate of individual leaf (A, C, and E) and declining trend of relative elongation rate (B, D, and F) in Swiss chard plants with red (A-B), pink (C-D), and yellow (E-F) petioles.

Leaf elongation and widening were halted at the same day. The slopy declining patterns of the midrib elongation were relatively similar to that of leaf blade widening, although the values of the two rates were different (Figure 5).

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Leaf area estimation model is a prerequisite before the leaf expansion rate (LER) can be calculated based on LA measured non-destructively on each individual leaf. Most of the cases for the regular non-compound leaf, LA can be accurately estimated ($R^2 > 0.95$) using the zero intercept linear regression and L×W as predictor. Results in Figure 6 confirmed that combination of the chosen regression model and the selected predictor were accurate in estimating LA of all three Swiss chard cultivars, i.e. red-, pink-, and yellow-petiole cultivars.

Flattened S-curve was barely recognized in LA increased during 16-day period starting after the young leaf was naturally unfolded (Figure 7). LA of the yellow-petiole cultivar was much higher than those of red- and pink-petiole cultivars. Average calculated-LA of red-, pink, and yellow-petiole cultivars were 825.16 cm^2, 941.10 cm^2, and 1252.65 cm^2, respectively. Variability in leaf size increased as the plants grew older.

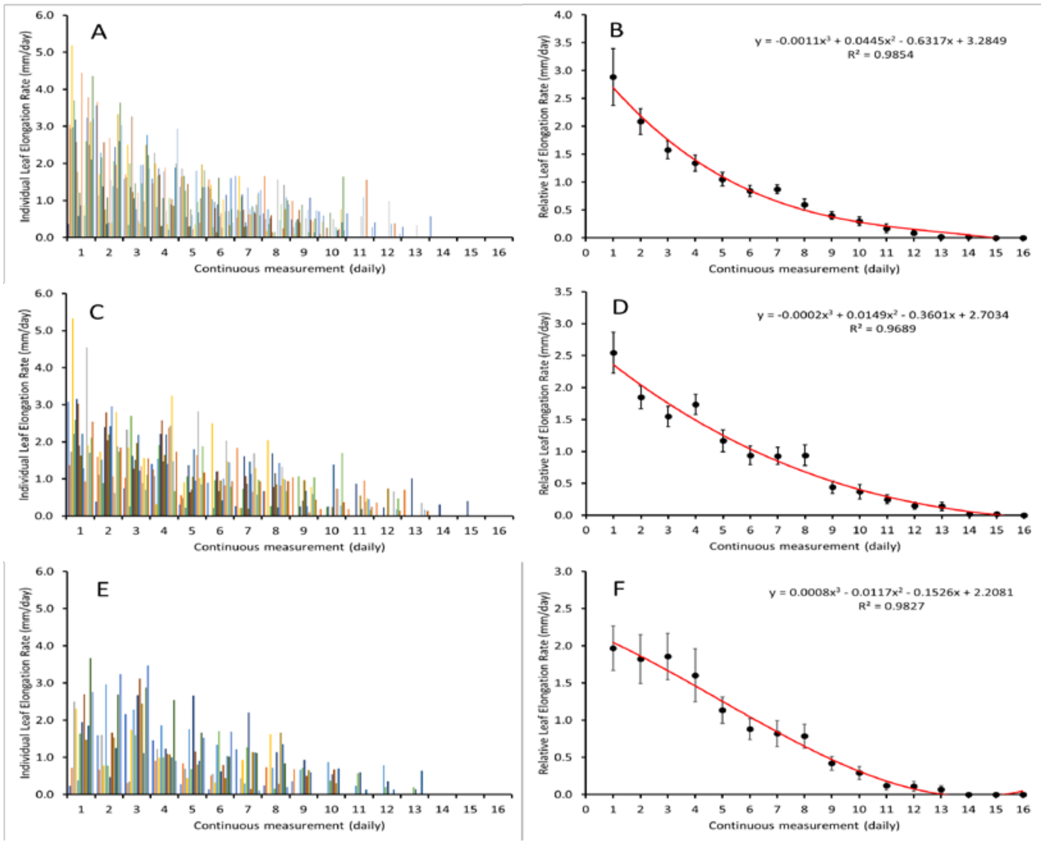

Figure 5. Widening rate of individual leaf (A, C, and E) and declining trend of relative widening rate (B, D, and F) in Swiss chard plants with red (A-B), pink (C-D), and yellow (E-F) petioles.

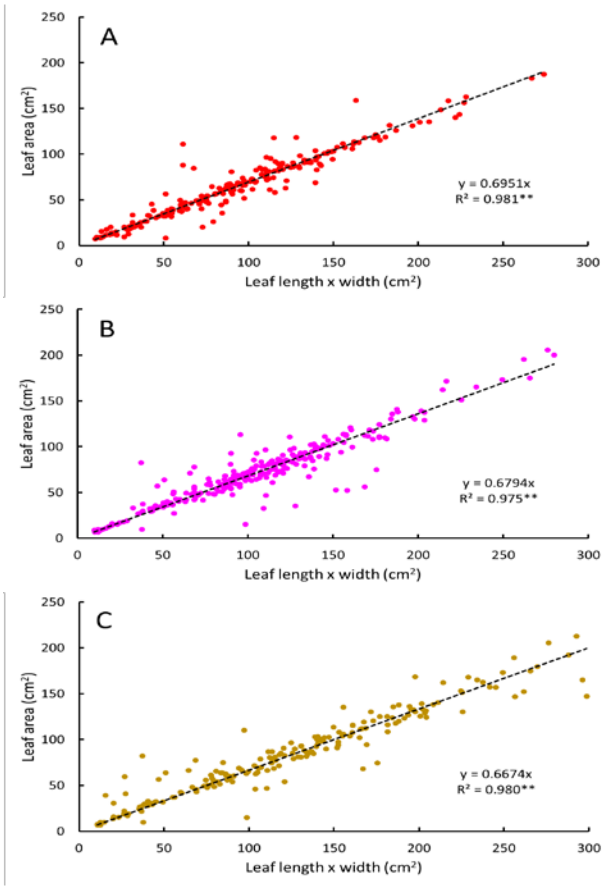

Figure 6. Leaf area estimation using L×W as predictor and the zero-intercept linear regression as selected model for Swiss chards with red (A), pink (B) and yellow (C) petioles.

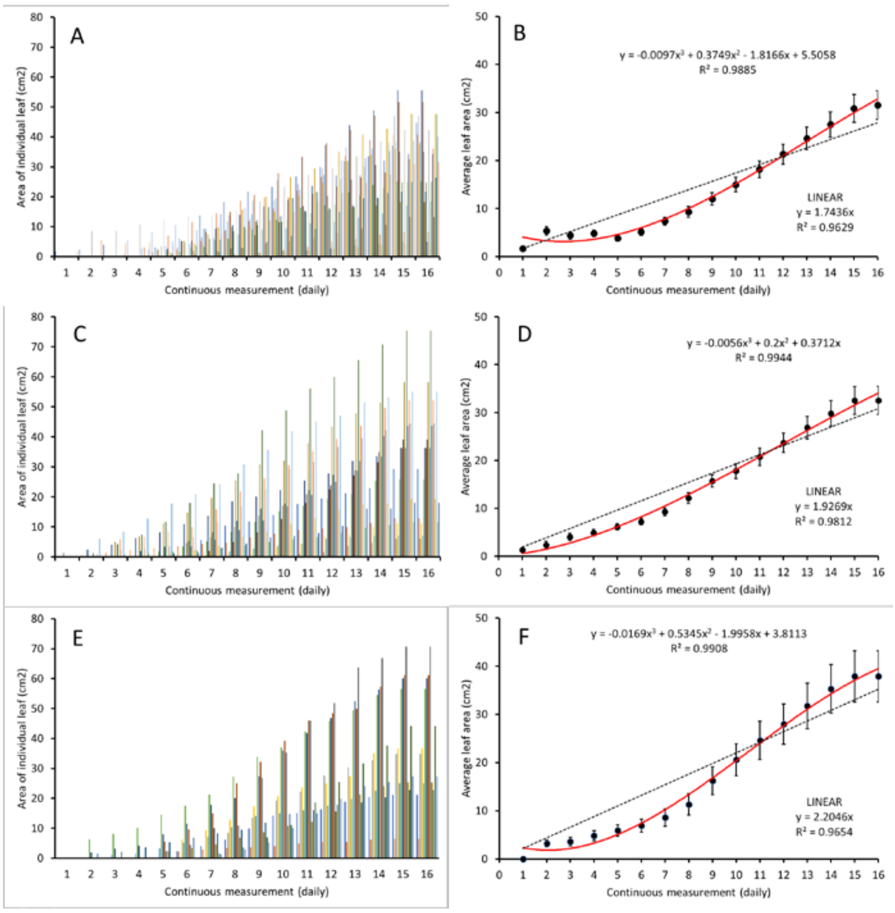

Figure 7. Leaf area of individual leaves (A, C, E) and average leaf area of Swiss chard (B, D, F) in red (A,B), pink (C, D) and yellow (E, F) petiole cultivars.

4. Discussion

4.1. Number of days to reach the maximum leaf size

Most studies related to leaf enlargement do not consider that the process takes place in two directions, which is formed from a combination of increasing the length and width of the leaves. In this study, differences due to lengthening were distinguished by the influence due to widening, because the ratio of length/width indicated whether the shape of the leaves was static or changed during the leaf enlargement process.

Nelissen *et al*. (2016) reported that leaf growth was temporally regulated with cell division ceasing earlier at the tip and continuing longer at the base of the leaf which may altered leaf shape. Leaf growth was enhanced by self-regulating pathways but affected by increase in cell number and growing conditions. The final size of leaf was closely controlled by environmental and genetic factors (Gonzalez *et al*., 2010). The growth rate started to decline soon after the division zone regressed. Despite leaf size differences at time of unfolding, the leaves of common bean (*Phaseolus vulgaris*) attained their full size in approximately 9 days (Lakitan *et al*., 2018a).

4.2. Leaf growth rate

Leaf growth was fluctuated during each 24-hour period. Relative leaf expansion rate (RLER) at night was significantly higher than daytime RLER in bean (Lakitan *et al*., 2018a) and tomatoes (Meihana *et al*., 2017). Higher nocturnal RLER was associated with higher leaf water content due to lower transpiration rate. Daytime leaf elongation rate (LER) was mainly temperature-dependent. LER at night was influenced by temperature and relative humidity (Stuerz and Asch, 2021). Leaf widening revealed a strong sensitivity to plant intercepted light and weak reaction to evaporative demand. Leaf elongation was receptive to evaporative demand and was not affected by the intercepted light (Lacube *et al*., 2017). Scoffoni *et al*. (2016) found the core for synchronization of hydraulic and photosynthetic physiology. Their reliance on climate confirms the function of water transport in photosynthetic activities.

Smithers *et al*. (2019) argued that plant growth was a highly mechanical process. Mathematics could provide an underlying framework for probing the fundamental unrevealed mechanisms and concluded that interdisciplinary collaborations were vital for tackling the challenge to understand plant growth mechanics. A similar argument was brought up by Katifori (2018) that the moves towards unraveling the problems of plant physiology and ecology could only be accomplished with collaboration amongst scientists in biology, computer science and physics.

Armon *et al*. (2021) identified that a growing leaf was a prototypical active solid and the cells were its active units. During the out-of-equilibrium process of cellular growth, the leaf increased its area, yet maintained a proper flat shape. Moreover, leaf growth strongly fluctuated in time and position. Growth isotropy did not homogenize in time. Mechanical analysis indicated that a leaf could stay flat only if the fluctuations were regulated. How this was achieved in the lack of a central control was unknown.

4.3. Leaf shape

Leaf shape is only a common concern in plant species identification and generally leaf shape is assumed to be static, although in fact in some types of plants the shape of the leaves sometimes changes during the leaf development process. In this study, leaf shape in Swiss chard did not change during leaf development. In agronomic and physiological studies, leaf deformation rarely receives attention.

Li *et al*., (2020) observed that there was significant spatial and temporal intraspecific variation in leaf size and shape in response to climate. Leaf size changing over time was positively correlated with precipitation, whereas leaf shape changes were mostly correlated with temperature. Quantitative models apprehending genetic diversity and environmental plasticity required understanding on the evolution and development of the leaf shape and its response to environmental stresses (Chitwood and Sinha, 2016).

Odiyi and Eniola (2015) reported that acid rain caused leaf morphological alterations, including leaf folding. Kumar and Pandey (2017) reminded that the high levels of UV-B radiation augmented total leaf abnormality. However, these changes might be temporal. Mahdid et al. (2021) described that leaf growth was recovered after termination of an abiotic stresses. The recovery rate was linked to the propensity of osmotic adjustment in the elongation zone. Furthermore, Qi *et al*. (2017) argued that mechanical heterogeneity of the cell wall within tissue might underlie organ shape asymmetry which might include alteration of the leaf shape.

4.4. Leaf area estimation

Estimation of LA is commonly done for further use in non-destructive continuous data collection for calculating absolute and relative leaf expansion rate respectively and other LA-based secondary morphological traits, including continuous measurement of specific leaf area, specific leaf weight. LA estimation was using length of midrib (L), width of leaf blade (W), or L×W as predictor. Several regression models had been evaluated for their accuracy in estimating LA. For single regular-shape leaf, such as Swiss chard leaf, the zero-intercept linear regression has been proven to be a very accurate LA estimator if L×W is used as predictor (Lakitan *et al*., 2018b; Widuri *et al*., 2017). Result of this study confirmed that combination of the zero-intercept linear regression and L×W as predictor was an accurate LA estimation.

5. Conclusion

Leaf is the most important organ in leafy vegetable, including Swiss chard; therefore, leaf growth rate and reliable information on how long it takes for a leaf to reach its full size are beneficial for farmers on deciding the best time to harvest the leaf. Monitoring leaf growth rate should be non-destructively done. Length of midrib, width of leaf blade, and combination of these two morphological traits (L×W) can be used as reliable predictors for continuous leaf area (LA) estimation. For single-blade and regular-shape Swiss chard leaves, highly accurate estimation model was achieved by using the zero intercept linear regression and L×W as the predictor. The slopes were 0.695, 0.679, and 0.667 for red, pink, and yellow petiole cultivars, respectively. Based on the estimated LA, it was found that the yellow-petiole cultivar grew significantly faster than both red and pink cultivars. However, the leaves of all cultivars reached their full size at around 12 days after the tiny young leaf blade was unfolded. It is recommended that Swiss chard leaves should be regularly harvested within 12 days after the unfolded immature leaf blade has been recognizable, i.e. at midrib length of about 1 cm.

Acknowledgement

High gratitude goes to the editor-in-chief of the Jordan Journal of Biological Sciences and all supporting staff. We also would like to deeply appreciate the comments and suggestions given by the anonymous reviewers of our manuscript. This research was funded by Indonesian Ministry of Education, Culture, Research, and Technology, Grant No. 057/E5/PG.02.00.PT/2022.

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