International Journal of Agricultural Technology 2024 Vol. 20(5):2137-2156
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Uncovering leaf chlorophyll, SPAD, and RGB coherence with the growth of Tatsoi (*Brassica rapa* subsp. *narinosa*) under nutrient and water deficiency

Setiawati, D. A.[1,2], Sutiarso, L.[1*], Ngadisih.[1] and Murtiningrum.[1]

[1]Department of Agricultural and Biosystems Engineering, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia; [2]Department of Agricultural Engineering, Faculty of Food and Agroindustrial Technology, Universitas Mataram, Mataram, Indonesia.

Setiawati, D. A., Sutiarso, L., Ngadisih and Murtiningrum (2024). Uncovering leaf chlorophyll, SPAD, and RGB coherence with the growth of Tatsoi (*Brassica rapa* subsp. *narinosa*) under nutrient and water deficiency. International Journal of Agricultural Technology 20(5):2137-2156.

Abstract The results showed that under extreme nutrient deficiency, yet with optimum watering duration (24 hours), Tatsoi experienced a reduction in leaf SPAD value (56.96%), directly correlated with decreased chlorophyll content (53.97%) and blue (B) value (37.62%). SPAD value was inversely proportional to the red (R) and the green (G) index, with an increase of 36.85% and 34.74%, respectively. The best-fitted model was the multiple linear regression using three variables of RGB color index to predict leaf SPAD value (R^2 = 0.911) and chlorophyll content (R^2 = 0.9698). Similar stress characteristics were found to be consistent at different watering durations. However, it was observed that nutrient stress had a more significant effect on Tatsoi growth parameters than water stress. The most affected growth parameter was the plant weight, with an average 85.09% reduction due to the decreasing photosynthesis rate of 23.61% and transpiration rates of 13.23% of all treatments. The correlogram analysis further showed that nutrient solution concentration (TDS) positively correlated with SPAD and chlorophyll content with correlation values of 0.97 and 0.94, respectively. The majority of Tatsoi growth parameters, except for root length, had high correlation values with TDS (> 0.9).

Keywords: Chlorophyll, Nutrient and water deficiency, RGB index, SPAD, Tatsoi

Introduction

Tatsoi (*Brassica rapa* subsp. *narinosa*) is a leafy vegetable recently recognized as a mixed salad composition. However, common cooking methods such as boiling, microwaving, steaming, and stir-frying reduce some beneficial nutrient compounds (Yang *et al*., 2019). Numerous people consume Tatsoi daily, specifically in urban areas, as it contains beneficial compounds for maintaining health. The nutritional substantial metabolite consists of amino acids, sugars, essential minerals, vitamins (A, B9, E, and K1), and glucosinolates (Zou *et al*., 2021).

[*]**Corresponding Author**: Sutiarso, L.; **Email:** lilik-soetiarso@ugm.ac.id

To supply the increasing market demand, urban farmers must provide sufficient quantity, quality, and continuity of vegetables. Due to limited productive land, vegetables have recently been cultivated using the hydroponic method on soil-less cultures, including Tatsoi. Although hydroponics highly depends on water availability, it could be applied to dry areas where water is rare. Using this method, Tatsoi or other crop production, specifically in tropical regions, will not be affected by the water shortage, during prolonged dry seasons due to the El Nino phenomenon (Pascual *et al*., 2018).

Tatsoi cultivated using the hydroponic method might potentially experience water stress. The cultivation of plants under limited water availability commonly leads to a reduction in crop production. Water stress adversely impacts many aspects of plant physiology, specifically photosynthetic capacity (Osakabe *et al*., 2014). Plant growth is negatively affected by reducing xylem and phloem inflow (Morandi *et al*., 2014). Moreover, water stress was reportedly responsible for a significant reduction in the amount of linolenic acid and elevated glucosinolates in oil (Saeed *et al*., 2016).

Aside from water stress, Tatsoi might experience nutrient stress, where extremely low or high concentrations of hydroponic nutrient solution could enhance antioxidant enzyme activities that suppress the growth and quality of the plant (Ding *et al*., 2018). Previous studies found that nutrient stress affected plants resulting in the reduction of leaf number, width, and area (Lee *et al*., 2018). Some plants experienced a decrease in root length and biomass but an increase in root length per shoot biomass and root-to-shoot ratio (Lopez *et al*., 2023).

Leaf chlorophyll content is also affected by water or nutrient stress. For most leaf, green-related colors are sourced from chlorophyll a and b (Shibghatallah *et al*., 2013). Based on a previous report, plants treated with higher nutrients had increased leaf chlorophyll content (Shah *et al*., 2017). Given that leaf color is proportionately correlated with nitrogen content, the detection is essential for nutrient application guidelines (Tao *et al*., 2020).

Conventional measurement of leaf chlorophyll is usually conducted by a destructive approach, where leaf pigment will be collected using organic solvent extraction and then analyzed photometrically (Limantara *et al*., 2015). The non-destructive method of measuring leaf chlorophyll through a SPAD meter is increasingly used due to the low cost, ease of estimation, and accuracy (Valverde, 2021). The relation between the result of the extraction method and SPAD meter has been reported to be relatively high (Wang *et al*., 2005). However, the accuracy depends on the thickness and morphology of the leaf surface (Limantara *et al*., 2015). Calibration is also required to provide an absolute quantity that is comparable between different studies and species (Brown *et al*., 2022).

Another alternative non-destructive method is based on RGB components analysis of digital images. Previous studies analyzed the chlorophyll content of various crops, such as *Arabidopsis* (Liang *et al*., 2017), barley (Guendouz *et al*., 2022), eucalyptus (Valverde, 2021), grapevine (Bodor-Pesti *et al*., 2023), pomegranate (Özreçberoğlu and Kahramanoğlu, 2020), quinoa and amaranth (Riccardi *et al*., 2014) using this method. Generally, the results showed high accuracy in measuring leaf chlorophyll content.

There have been limited studies that investigated coherence between leaf chlorophyll, SPAD index, and RGB value with the growth of Tatsoi under abiotic stress. Therefore, this study aimed to determine the coherence between changes in leaf RGB with Tatsoi stress characteristics. The applicability of RGB value to detect stress in Tatsoi under insufficient nutrient and water availability was also evaluated.

Materials and methods

Experimental schedule and location

This study commenced from November to December 2022 during the rainy season. Totally, an installation of a 9 m^2 nutrient film technique (NFT) hydroponic module was used in the experiment. The module was located in a naturally ventilated tropical greenhouse in the Faculty of Agriculture, Universitas Gadjah Mada, at Depok Sub-district, Sleman Regency, Yogyakarta, Indonesia (7°46'06" S, 110°22'54" E).

Experimental design and layout

Tatsoi used in this study was plant aged 10 days after sowing at 3 cm x 3 cm x 3 cm cubical Rockwool. The grown seedlings with 2.4 ± 0.1 cm height were then transplanted individually on a net pot placed at the NFT hydroponic module. As replications, 8 plants were used for each treatment, with Figure 1 showing the design of the hydroponic module and the top view in the greenhouse.

There were three variations of nutrient concentration with three different watering durations, including 24, 12, and 6 hours. Therefore, a total of nine treatments were conducted. Different AB mix nutrient concentrations were applied to the plant at each growth phase based on Total Dissolved Solid (TDS) value, including high (A0, 100%), moderate (A1, 75%), and low nutrient (A2, 37.5%) concentration as shown in Table 1. The nutrient used was AB Mix from Purie Garden Jogjakarta with chemical composition of N-total (8%), P_2O_5 (7%), K_2O (7.5%), CaO (10%), Mg (3%), Mn (300 ppm), B (200 ppm), Fe (600 ppm), Zn (100 ppm), Cu (40 ppm), and Mo (10 ppm). Every two days, the TDS value of the nutrient solution in the reservoir tank of each treatment was measured and adjusted manually at the appropriate growth stage. According to a previous study, the recorded TDS value of NFT hydroponic nutrient solution inside a naturally ventilated greenhouse changes over time due to air temperature fluctuation (Hamsah *et al*., 2023).

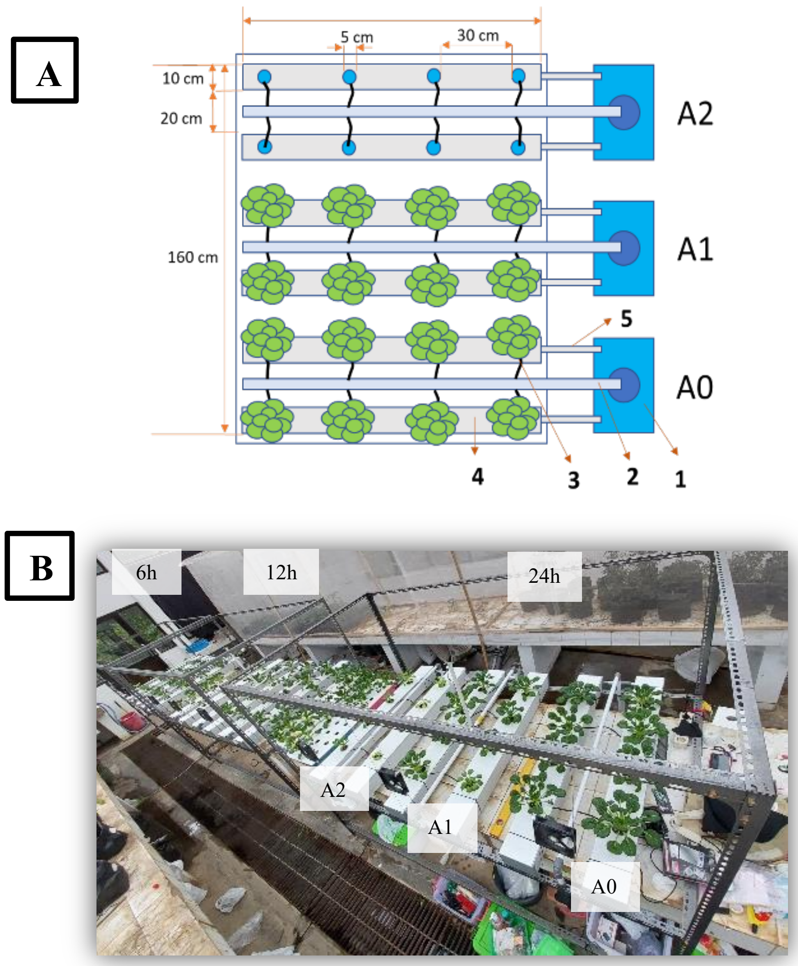


Figure 1. The design of the hydroponic module for one treatment (A); Top view of the hydroponic module for each treatment (B).
Note. 1 = nutrient reservoir, 2 = inlet pipe, 3 = emitter, 4 = gulley, 5 = outlet pipe

Table 1. Average nutrient variation for each treatment

Treatment	TDS of AB Mix nutrient concentration (ppm)		
	Initial stage	**Mid-stage**	**Final stage**
A0 (High)	800	1000	1200
A1 (Moderate)	600	800	1000
A2 (Low)	300	400	500

Study parameters

The parameters in this study were mainly collected using nondestructive methods to observe continuous plant characteristics during stress. These included chlorophyll content, SPAD index, and RGB value of Tatsoi leaf. Plant growth, metabolism comprising photosynthesis, and transpiration rate, alongside microclimate in the greenhouses, were also observed as supporting data.

The Leaf SPAD index was measured every two days using a chlorophyll meter (SPAD-502 Konica Minolta®). Chlorophyll content from each treatment was measured with the solvent extraction method and analyzed further using a spectrophotometer. Photographs of the plant leaf canopy taken using Easy Leaf Area (ELA) were assessed through ImageJ (NIH, Bethesda, MD, USA) software to quantify the values of the red (R), green (G), and blue (B) colors index.

A portable photosynthesis system (IRGA, LI-6400, LI-COR, Inc., Lincoln, NE, USA) was used to assess the rate of photosynthesis and leaf transpiration during the peak vegetative period. The canopy area was measured using ELA application on an Android OS mobile phone based on a previous study (Setiawati *et al*., 2023b).

Plant height and root length were measured manually every two days, while the wet weight was assessed at harvest using a digital scale (i2000 Taffware Digipounds). The microclimate data were recorded automatically per minute on the Arduino UNO R3 microcontroller equipped with a data logger, sensors (DHT22 and GY49), and LCD (20x4) (Setiawati *et al*., 2023a).

The collected data were then represented as time series graphs to examine parameter variations during the experiment. Chlorophyll, SPAD, and RGB data were analyzed using a multiple linear regression approach. The data of plant growth were analyzed using R Studio Software, with a two-way analysis of variance (2-way ANOVA) method used to determine the significance. Subsequently, the Tukey HSD (honestly significant difference) test was applied for further post-hoc analysis. A correlation analysis method was used to explore the relationships between different parameters.

Results

Microclimate data

Monitoring changes in microclimate data inside the greenhouse was vital as it directly affected Tatsoi's growth. Microclimate data within the greenhouse during the experiment is shown in Table 1. The highest air temperature occurred at noon (31.8°C), inversely with the relative humidity found to be the least (68.40%). However, the sunlight intensity at noon was lower (11,538.61 lux) compared to the morning (15,122.61 lux). The highest relative humidity was found in the evening (79.9%), with the lowest sunlight intensity (< 2000 lux).

Tabel 2. Average microclimate data inside the greenhouse (Nov – Dec 2022)

Parameter	Morning	Noon	Evening
Temperature (°C)	30.97	31.87	28.09
RH (%)	70.23	68.40	79.93
Intensity (lux)	15,122.61	11,538.61	1,917.33

Leaf color RGB, SPAD, and chlorophyll

One method to determine Tatsoi stress is observing leaf color changes related to chlorophyll content. The observational data on SPAD readings up to 27 DAP (days after planting) in the experiment is shown in Figure 2. Additionally, data of SPAD, RGB, and chlorophyll content at harvest time for each treatment is shown in Figure 3.

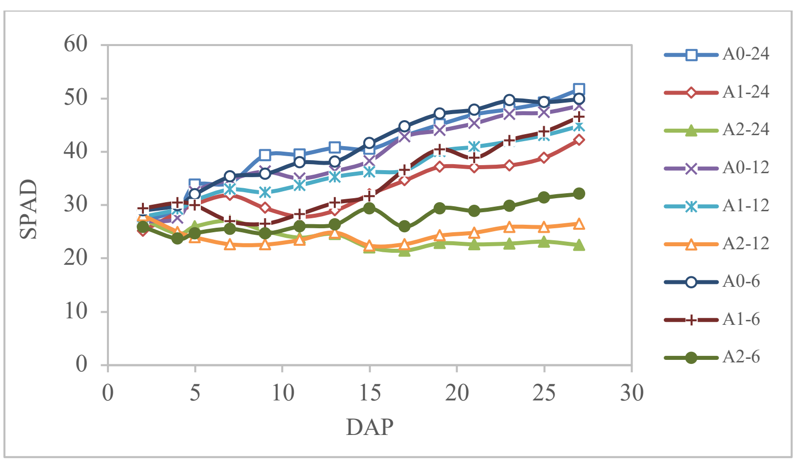


Figure 2. Change of Tatsoi leaf SPAD value until 27 DAP of each treatment

A consistent pattern where SPAD values of the Tatsoi leaf increased as the plant grew (Figure 2). The treatments that received high nutrient concentrations consistently had the maximum SPAD values, regardless of the watering duration, including A0-24, A0-12, and A0-6. The values were 51.34, 50.43, and 52.4, respectively. This pattern corresponded with the highest chlorophyll content observed at harvest time (Figure 3), reaching 1.24 $g.mg^{-1}$, 1.25 $g.mg^{-1}$, and 1.37 $g.mg^{-1}$. In contrast, SPAD and chlorophyll values indicated an inverse correlation with R and G values, directly correlated with B.

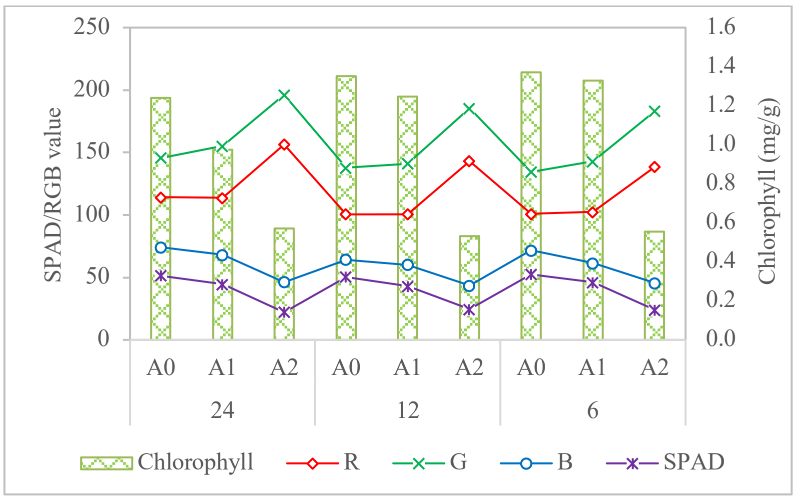
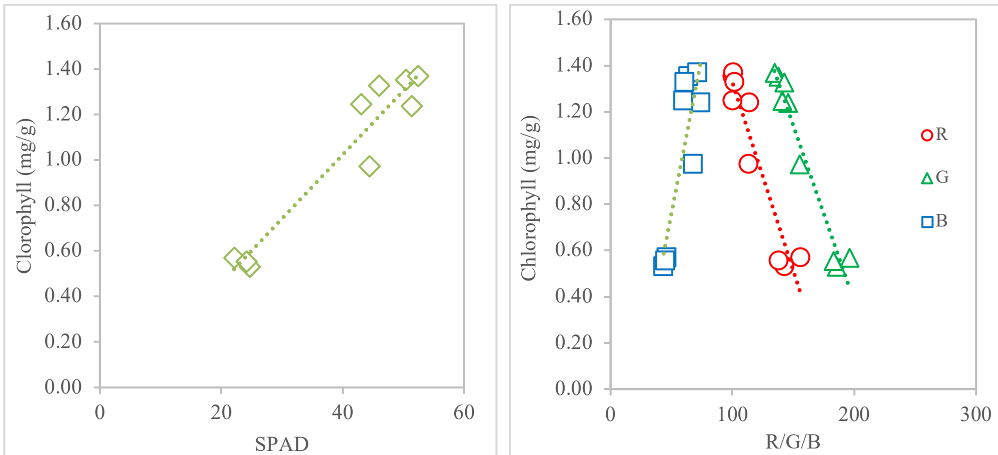


Figure 3. Comparison between leaf's chlorophyll content and SPAD/RGB value after harvest

The analysis result showed that under extreme nutrient deficiency, Tatsoi experienced a reduction in leaf SPAD value (56.96%), directly correlated with low chlorophyll content (53.97%) and blue (B) value (37.62%). SPAD value was inversely proportionate with R and G values, of 36.85% and 34.74%, respectively. It showed that the lesser the chlorophyll, the higher the R and G values (Figure 3).

The multiple linear regression analysis proved that a strong correlation exists between SPAD value and chlorophyll content ($R^2 = 0.9203$), as shown in Figure 4 (a). Similarly, the relationship between the individual color index (R, G, and B) and SPAD was strong, based on the determination coefficient values (R^2) of 0.8544, 0.9347, and 0.9152, respectively, as shown in Figure 4 (b). The determination coefficient of the multiple linear regression analysis obtained values of 0.968, 0.9821, 0.9896, and 0.9911 for SPAD (R, G), (R, B), (G, B), AND (R, G, B), respectively. These coefficients underscore the significant relationships among the variables. Further analysis showed a strong relation between chlorophyll content and RGB index, indicated by a high determination coefficient ($R^2 > 0.9$) except for R index (Table 3), and B index (Table 4).



(a) (b)

Figure 4. Correlation graph between chlorophyll content with SPAD value (a) and SPAD value with RGB value (b) of Tatsoi leaf

Table 3. Equations, coefficient of determination (R^2), and p-value for the model of SPAD and RGB index based on multiple linear regression

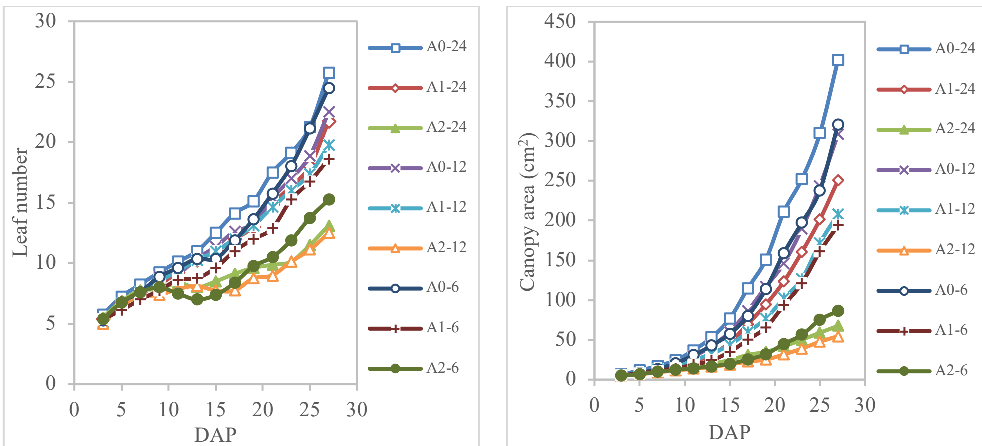
Color index	Equation	R-squared	p-value
R	SPAD = 104.14416 - 0.54089*R	0.8544	0.00036
G	SPAD = 120.95094 - 0.51359*G	0.9347	2.13E-05
B	SPAD = -21.4651 + 1.0311*B	0.9152	5.35E-05
RG	SPAD = 135.8120 + 0.6581*R - 1.1031*G	0.968	3.27E-05
RB	SPAD = 31.54676 - 0.25588*R + 0.65114*B	0.9821	5.69E-06
GB	SPAD = 55.79512 - 0.29389*G + 0.51230*B	0.9896	1.13E-06
RGB	SPAD = 69.2903 + 0.1848*R - 0.4908*G + 0.4390*B	0.9911	1.52E-05

Table 4. Equations, coefficient of determination (R^2), and p-value for the model of Chlorophyll and RGB index based on multiple linear regression

Color index	Equation	R-squared	p-value
R	Chl = 2.9565 - (0.0163*R)	0.9025	8.76E-05
G	Chl = 3.4256 - (0.0152*G)	0.9576	4.66E-06
B	Chl = -0.5997 + (0.0272*B)	0.7423	0.00283
RG	Chl = 3.6542 + (0.0101*R) - (0.0243*G)	0.9667	3.68E-05
RB	Chl = 1.9935 - (0.0125*R) + (0.008637*B)	0.9287	0.00036
GB	Chl = 3.2573 - (0.0147*G) + (0.0013*B)	0.9580	7.42E-05
RGB	Chl = 4.367 + (0.0151*R) - (0.030867*G) - (0.004704*B)	0.9698	0.00032

Leaf canopy area and leaf number

Tatsoi is typically consumed for leaf, hence, monitoring changes in this part is essential as a stress indicator. The observed changes over 27 DAP during various nutrient and watering durations are shown in Figure 5.

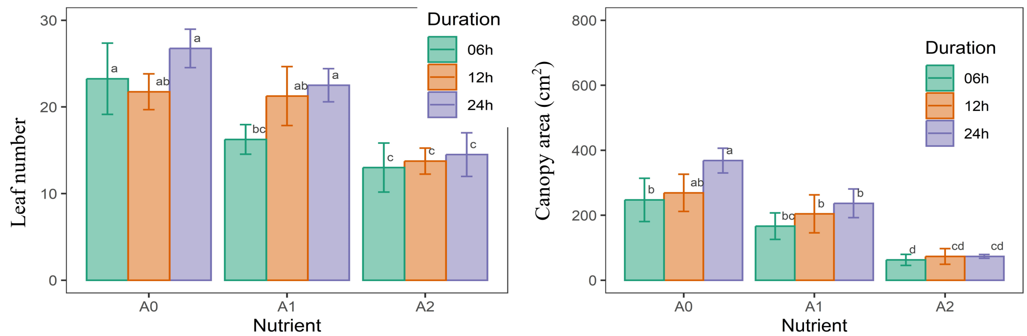


(a) (b)

Figure 5. Leaf number (a) and canopy area (b) change of Tatsoi under various treatments

The results showed consistent leaf number and canopy area growth with time across all treatments. On average, the lowest leaf number and canopy area change rates were found in treatment with extreme nutrient stress (A2), of 0.42 leaf.day^{-1} and 2.69 cm^2.day^{-1}, respectively. Conversely, the highest rates were observed in the control treatment (A0), with 0.79 leaf.day^{-1} and 14.08 cm^2.day^{-1} of change.

The highest leaf number (26 leaf) and canopy area (401.856 cm²) were observed in the treatment with the highest nutrient concentration and a 24-hour watering duration (A0-24). The 75% nutrient concentration (A1-24) had a milder impact, causing a slight decrease in leaf count and canopy area to 15.53% (22 leaf) and 37.71% (250.321 cm²), respectively. Furthermore, the 37.5% nutrient concentration (A2-24) corresponded to a decrease in both leaf count and canopy area to 49.03% (13 leaf) and 83.19% (67.554 cm²), respectively. The pattern of declining leaf count and canopy area was also found in different watering durations (6 and 12 hours). These changes indicated that Tatsoi experienced stress due to the reduction in nutrients.



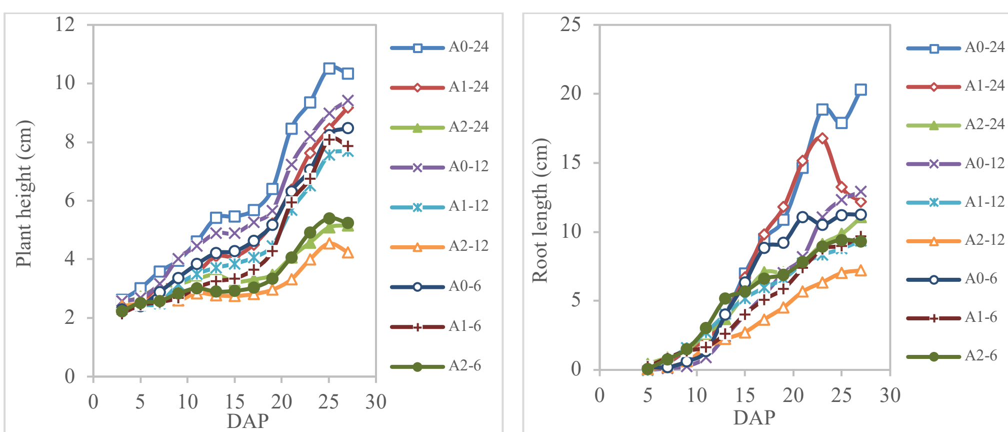
(a) (b)

Figure 6. Average leaf number (a) and canopy (b) at various treatments after harvested (27 DAP)

The average leaf number and canopy area data for each treatment at harvest time are shown in Figure 6. The two-way ANOVA results indicated that nutrient reduction significantly affected plant leaf number and canopy area ($P < 0.05$). The post-hoc using the Tukey HSD test revealed that water stress had a less significant effect than nutrient stress on leaf number and canopy area at all treatments.

Plant height and root length

Tatsoi height and root length growth showed similar trends with leaf count and canopy area. The plant height and root length gradually increased across all treatments (Figure 7).

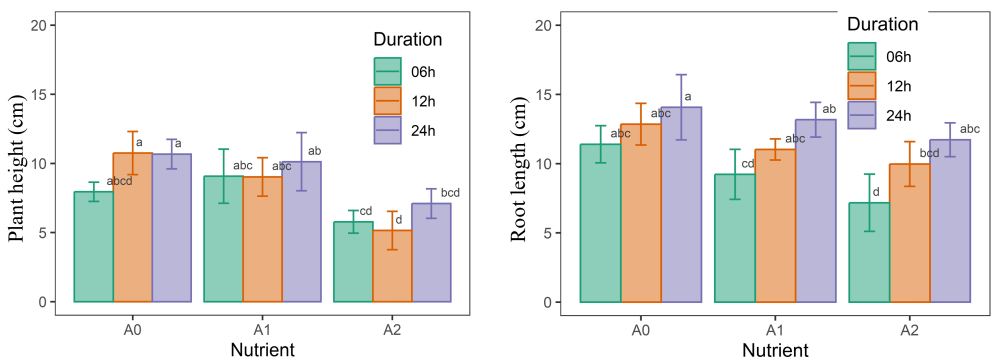


(a) (b)

Figure 7. Plant height (a) and root length (b) change of Tatsoi under various treatments

The treatment with the lowest nutrient content (A2, 37.5%) had minimum growth rates, with plant height and root length increasing by 0.23 cm.day^{-1} and 0.85 cm.day^{-1}. Maximum growth rates were found in the treatment with the highest nutrient content (A0, 100%), with plant height and root length increasing by 0.56 cm.day^{-1} and 1.41 cm.day^{-1}, respectively.

The control treatment, with sufficient water and the highest nutrient concentration (A0-24) yielded the maximum plant height and the most extended root length of 10.34 cm and 20.33 cm. The 75% nutrient concentration treatment (A1-24) had a milder impact, causing a slight decrease of 15.38% (9.84 cm) and 44.21% (12.19 cm). The extreme nutrient stress (A2-24, 37.5%) caused a reduction to 50.54% (5.75 cm) and 54.21% (11.06 cm), respectively.



(b) (d)

Figure 8. Average plant height (a) and root length (b) at various treatments after harvest (27 DAP)

The observed changes in plant height and root length show that Tatsoi experienced stress due to reduced nutrient availability. In addition, both experimental rounds demonstrated similar patterns regardless of the watering durations (6 and 12 hours). The average plant height and root length data at harvest time for each treatment were further analyzed using a two-way ANOVA, as shown in Figure 8. Based on the results, the nutrient and water reduction combination significantly influenced plant height and root length ($P < 0.05$). The post-hoc using the Tukey HSD test revealed that water stress had a less significant effect on plant height and root length than nutrient stress at all treatments.

The appearance of plant height and root length at harvesting is shown in Figure 9. Tatsoi responded to nutrient and water stress by reducing leaf number, canopy area, plant height, and root length. Morphologically, the stress could be detected by leaf color turning lighter green or even yellow.

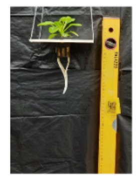
Watering duration	Nutrient		
	A0	A1	A2
24 h			
12 h			
6 h			

Figure 9. Tatsoi height and root length at various treatments

Biomass yield

The changes in leaf number, canopy area, plant height, and root length tend to directly affect biomass yield at the harvesting time. The average fresh weight of Tatsoi harvested in the experiment is shown in Figure 10.

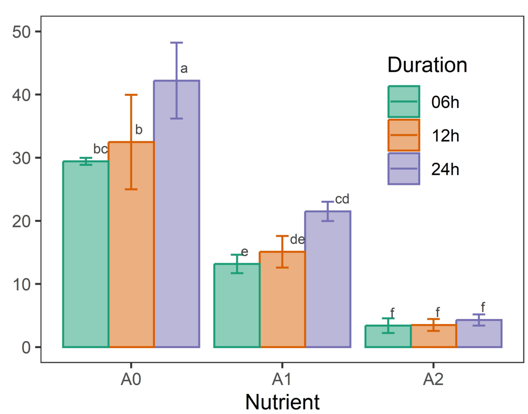


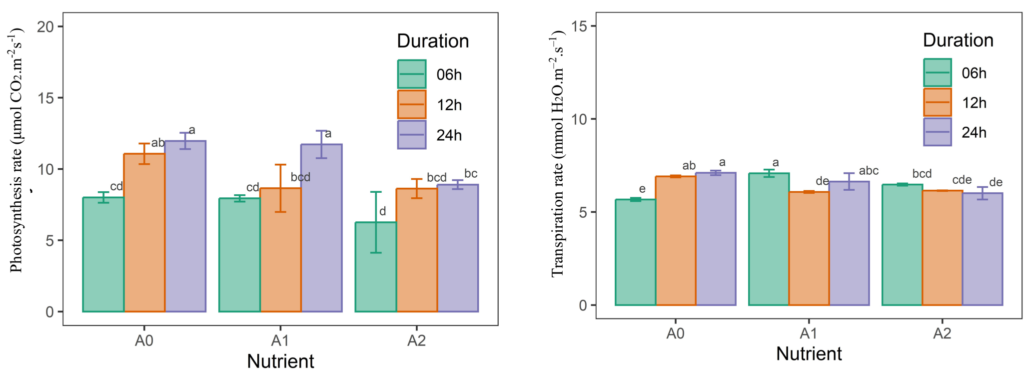
Figure 10. Average plant weight at various treatments after harvest (27 DAP)

Tatsoi weight showed a slight decreased, amounting to 40.15% and 85.09%, due to low nutrient concentration treatment of A1 (75%) and A2 (37.5%), respectively. The weight fluctuations indicated the occurrence of stress due to reduced nutrient availability. This pattern was consistent across all watering durations. Furthermore, the two-way ANOVA results showed that nutrient reduction and watering duration significantly affected plant weight ($P < 0.05$). The post-hoc using the Tukey HSD test revealed that water and nutrient stress significantly affected leaf number and canopy area except in the extreme nutrient stress.

Photosynthesis and transpiration rate

Plant growth relies on photosynthesis, which depends on light, transpiration, and water availability. Therefore, observing photosynthesis and transpiration rate changes can serve as crucial indicators of stress. The data on these parameters observed during the experiment is shown in Figure 11.

For all watering duration, the 75% (A1) and 37.5% nutrient (A2) treatment led to a decrease of 17.67% and 23.61% in the photosynthesis rate. The transpiration rate decreased only in the 24-hour and 12-hour watering durations, with reductions of 9.32% and 13.23%, respectively. Contrastingly, increasing transpiration was observed at the 6-hour watering durations in the A1 treatment. This study also showed that the photosynthesis rate decreased due to insufficient water at all nutrient treatments.



(b) (d)

Figure 11. Average photosynthesis and transpiration rate at various treatment

The two-way ANOVA results showed that nutrient reduction and watering duration significantly affected photosynthesis rate ($P < 0.05$). For transpiration rate, a significant effect occurred in the combination of the nutrient and watering duration (P < 0.05). The post-hoc using the Tukey HSD test revealed that water stress affected the photosynthesis rate more significantly than nutrient stress. The water stress affected the transpiration rate more than the nutrient stress in the optimum nutrient treatment (A0).

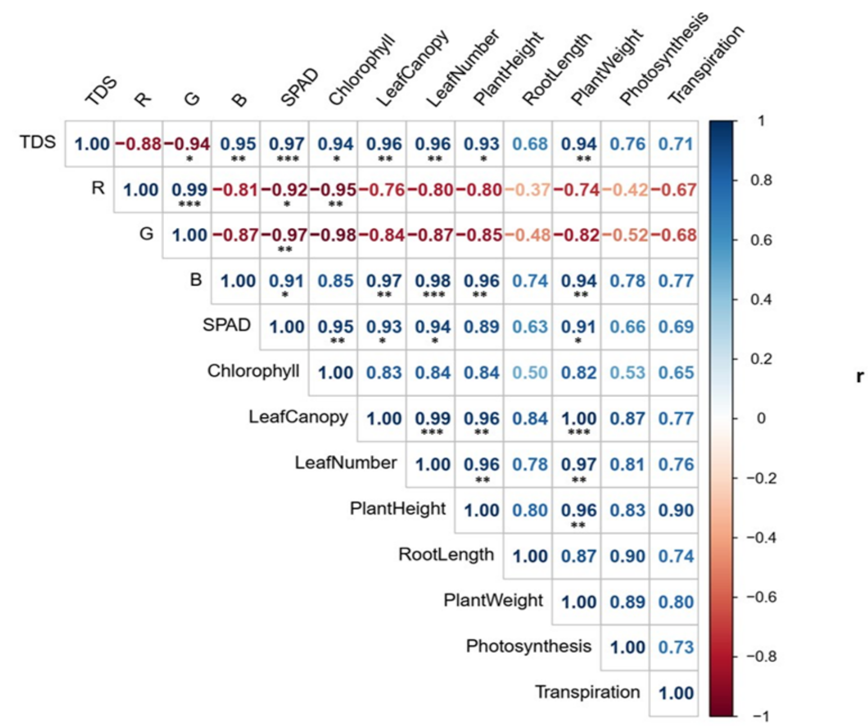


Figure 12. Correlogram plot of the observed parameter of all treatment
Note. *, **, *** Statistically significant at P < 0.05, 0.01, and 0.001; r = Correlation coefficient. The color intensity of the circle indicates the strength of the correlation between parameters. Non-star means not-statistically significant at P > 0.05. Blue and red indicate positive and negative correlations respectively.

Correlation analysis in Tatsoi stress

The nutrient solution concentration (TDS) is positively correlated with SPAD and chlorophyll content of Tatsoi leaf by correlation values of 0.97 and 0.94, respectively (Figure 12). The high correlation values were also found between TDS and morphological parameters (r > 0.9), except for root length (0.68). Meanwhile, TDS correlation values were 0.76 and 0.71 for photosynthesis and transpiration, respectively. R index color was inversely proportional and statistically significant to SPAD (P < 0.05) and chlorophyll (P

< 0.01) with r values of 0.92 and 0.95, respectively. G index color was also inversely proportional to SPAD (r = 0.97) and chlorophyll (r = 0.98), but statistically significant only for SPAD ($P < 0.01$). In contrast, B was the only index color directly proportional and statistically significant to SPAD (r = 0.91, $P < 0.05$). These similar patterns were also found in plant morphology and physiology, where only B index showed a direct proportionate relation.

Discussion

In this study, Tatsoi was cultivated inside a naturally ventilated tropical greenhouse, where the microclimate was highly influenced by changes in external conditions (Shamshiri and Ismail, 2013). The sunlight served as the primary energy source for plant photosynthesis, but it gradually increased the air temperature inside the greenhouse since some sunlight became trapped and could not escape. This explains why the maximum temperature occurred at noon (31.8°C). In contrast, lower sunlight intensity occurred at noon than in the morning during the rainy season because the cloud accumulation in the atmosphere attenuated the solar irradiance (Diaz-Torres *et al*., 2017) entering the greenhouse. High daily precipitation led to relatively high air humidity (60%-80%) assisted in optimizing the conditions for cultivating *Brassica* plant (Han *et al*., 2019).

Based on the results, the primary factor driving morphological and physiological changes under nutrient stress in Tatsoi was the reduction of chlorophyll content which could potentially be identified using RGB method. According to Table 3, the best-fitted model of multiple linear regression shown by the highest coefficient determination ($R^2 = 0.9911$) occurred when using three variables of RGB color index to predict SPAD value. A similar pattern occurred in the prediction of total chlorophyll content (Table 4), although with a lower coefficient of determination ($R^2 = 0.9698$). For SPAD model, G was negatively correlated while R and B were positively correlated. For chlorophyll model, G and B were negatively correlated, while R was positively correlated. These results differ from previous studies where R and G were negatively correlated with chlorophyll in amaranth and quinoa (Riccardi *et al*., 2014), as well as potato (Yadav *et al*., 2010) plant leaf.

Among the three color index in the single linear regression model, B color showed a negative correlation both with SPAD and chlorophyll, while R and G indicated a positive correlation. The best correlation was obtained by G color index with a coefficient of determination at 0.9347 and 0.9576 for SPAD and chlorophyll, respectively. Other studies that analyzed chlorophyll of pomegranate leaf showed different results, where R correlation was negative while G and B were positive (Özreçberoğlu and Kahramanoğlu, 2020). Therefore, this study confirmed that different plant species had different correlations between leaf chlorophyll and RGB index considered in identifying plant stress due to internal factors or external traits.

Tatsoi is characterized by a dark green color, which is typically associated with high chlorophyll content. Real-time change of chlorophyll in leaf could be detected using SPAD meter. The increasing SPAD index indicated the increasing chlorophyll content. The low leaf chlorophyll content (SPAD < 35) was found in plants with extreme nutrient treatment (A2) at all watering durations. The reduction of chlorophyll occurred mainly due to nutrient deficiency. This result corresponded with previous studies which found decreased pigment content per unit area, as well as in the total amount produced per plant under limited nutrient supply (Shah *et al*., 2017).

The results suggested that the low chlorophyll content was directly proportional to the low leaf nitrogen content and other macronutrient or micronutrient elements. Ding *et al*., (2018) reported that leaf-relative chlorophyll content significantly decreased in low-nutrient treatments due to deficiencies in essential elements for biosynthesis such as nitrogen (N), magnesium (Mg), and ferum (Fe). Furthermore, Veazie *et al*., (2020) stated that total chlorophyll concentrations were found to be lower in the leaf of plants deficient in nitrogen (N), iron (Fe), and manganese (Mn).

In this study, Tatsoi leaf subjected to extreme nutrient stress (A2) had light green coloration, accompanied by the lowest chlorophyll content, SPAD value, and B value, while R and G values were the highest. RGB regression analysis conducted in this study was consistent with the results of Liu *et al*. (2021), which examined barley leaf using SPAD chlorophyll meter and a mounted digital camera on an unmanned aerial vehicle (UAV). The coefficient of determination (R^2) between the measured and estimated SPAD values obtained from the linear and multiple regression models was lower (0.7929) compared to the results in this experiment (0.9911). This discrepancy could be attributed to differences in leaf characteristics between barley and Tatsoi plants.

The results of this experiment also were consistent with the observations made by Kartika *et al*. (2021), which studied Tatsoi leaf SPAD values in a conventional system. It was found that values gradually increased reaching 51.62 at 26 days after seedlings were transplanted (DAT) and then significantly decreased at 38 DAT. It indicated the process related to the synthesis, accumulation, and subsequent degradation of leaf chlorophyll. In this experiment, SPAD value gradually increased and reached approximately 51.34 for the control treatment (A0-24) at the time of harvest.

This study found that reduced chlorophyll content in Tatsoi leaf due to nutrient stress negatively affected the photosynthesis rate. The plant with extreme nutrient stress (A2, 37.5%) had the highest reduction of 23.61% due to the decreasing chlorophyll content of 55.99%. According to previous reports, chlorophyll is a pigment-form binding protein complex (LHC), which absorbs most of the light exposed to leaf (Kume *et al*., 2018). It functions in light-trapping and energy transduction during the anabolic process of photosynthesis (Nkcukankcuka *et al*., 2022). Other studies found that plants with strong nutrient deficiency showed suboptimal development of the photosynthetic machinery, affecting both PSII (photosystem II) and PSI (photosystem I) (Kalaji *et al*., 2018).

The reduction in the photosynthesis rate of plants resulted from lower chlorophyll content due to nutrient deficiency impacting morphological aspects including leaf number and canopy, plant height and root length (Figures 7 & 8), as well as weight. Basically, photosynthesis plays a crucial role in providing essential building blocks of plant cells through the light and dark cycle mechanism to convert carbon dioxide (CO_2) into sugar (glucose) and other products, namely amino acids and lipids (Johnson, 2016). Therefore, a reduction in the photosynthesis rate directly led to lowering in the production of essential elements necessary for maintaining plant growth and development. The synthesis of chromatin which regulates the vital plant growth and development processes (Ojolo *et al*., 2018) is also disrupted due to lower photosynthesis rate.

Correlogram analysis results showed a positive and significant correlation between nutrient solution concentration (TDS) and various plant morphological characteristics including leaf canopy, and number, plant height, and weight, root length. There was also a positive and significant correlation with physiological markers such as B index, and SPAD values, including leaf chlorophyll content. A previous study found that apart from insufficient sunlight, nutrient deficiencies in the growth medium can also adversely affect the photosynthetic process (Kalaji *et al*., 2014) and vice versa. However, based on this study results, stress due to nutrient deficiency was statistically insignificant in photosynthesis and transpiration, although the correlation value was relatively high ($r >= 0.71$). This condition might be related to other mechanisms driving photosynthesis and transpiration in plants, including leaf stomatal response under different water availability and sunlight intensity.

Previous studies reported that stomata density had a positive linear relation with water supply. In the short term, the more the water supply, the higher the stomata density leading to increasing transpiration of plant leaf (Putra *et al*., 2022). Another study stated that leaf canopy area of Tatsoi grown in less watering duration demonstrated an increasing trend in accordance with the growth period, suggesting the photosynthesis process still occurred despite the limited water availability (Setiawati *et al*., 2023a). In addition, stomata have been shown to respond positively to sunlight intensity, where a larger stomata area was found in the plant treated with full sunlight, enhancing faster gas exchange and photosynthesis (Putra *et al*., 2023).

Acknowledgments

The author is grateful to the Centre for Education Financial Services and the Indonesia Endowment Funds for the support provided in this study.

References

Bodor-Pesti, P., Taranyi, D., Nyitrainé Sárdy, D. Á., Le Phuong Nguyen, L. and Baranyai, L. (2023). Correlation of the Grapevine (*Vitis vinifera* L.) Leaf Chlorophyll Concentration with RGB Color Indices. Horticulturae, 9:1-9.

Brown, L. A., Williams, O. and Dash, J. (2022). Calibration and characterisation of four chlorophyll meters and transmittance spectroscopy for non-destructive estimation of forest leaf chlorophyll concentration. Agricultural and Forest Meteorology, 323:109059.

Diaz-Torres, J. J., Hernandez-Mena, L., Murillo-Tovar, M. A., León-Becerril, E., López-López, A., Suárez-Plascencia, C., Aviña-Rodriguez, E., Barradas-Gimate, A. and Ojeda-Castillo, V. (2017). Assessment of the modulation effect of rainfall on solar radiation. Meteorological Applications, 24:180-190.

Ding, X., Jiang, Y., Zhao, H., Guo, D., He, L., Liu, F., Zhou, Q., Nandwani, D., Hui, D. and Yu, J. (2018). Electrical conductivity of nutrient solution influenced photosynthesis, quality, and antioxidant enzyme activity of pakchoi (*Brassica campestris* L. Ssp. Chinensis) in a hydroponic system. PLoS ONE, 13:1-15.

Guendouz, A., Bendada, H. and Benniou, R. (2022). A Comparative Study of Chlorophyll Content Estimation in Barley (*Hordeum vulgare* L.) Genotypes Based on RGB (Red, Green, Blue) Image Analysis. Agricultural Science Digest, 42:68-71.

Hamsah, Sumarsono, J., Setiawati, D. A., Abdullah, S. H., Priyati, A. and Putra, G. M. D. P. (2023). Design of AB Mix Nutrition Control and Monitoring System. Proceedings of the 1st Unhas International Conference on Agricultural Technology (UICAT 2021), May.

Han, W., Yang, Z., Huang, L., Sun, C., Yu, X. and Zhao, M. (2019). Fuzzy comprehensive evaluation of the effects of relative air humidity on the morpho-physiological traits of Pakchoi (*Brassica chinensis* L.) under high temperature. Scientia Horticulturae, 246:971-978.

Johnson, M. P. (2016). Photosynthesis. Essays in Biochemistry, 60:255-273.

Kalaji, H. M., Bąba, W., Gediga, K., Goltsev, V., Samborska, I. A., Cetner, M. D., Dimitrova, S., Piszcz, U., Bielecki, K., Karmowska, K., Dankov, K. and Kompała-Bąba, A. (2018). Chlorophyll fluorescence as a tool for nutrient status identification in rapeseed plants. Photosynthesis Research, 136:329-343.

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., Cetner, M. D., Łukasik, I., Goltsev, V., Ladle, R. J., Da, P. and Ahmad, P. (2014). The Use of Chlorophyll Fluorescence Kinetics Analysis to Study the Performance of Photosynthetic Machinery in Plants. In Emerging Technologies and Management of Crop Stress Tolerance: Volume II - A Sustainable Approach (Vol. 2). Retrieved from https://doi.org/10.1016/B978-0-12-800875-1.00015-6

Kartika, K., Lakitan, B., Ria, R. P. and Putri, H. H. (2021). Effect of the cultivation systems and split fertilizer applications on the growth and yields of Tatsoi (*Brassica rapa* subsp. Narinosa). Trends in Sciences, 18. Retrieved from https://doi.org/10.48048/tis.2021.344

Kume, A., Akitsu, T. and Nasahara, K. N. (2018). Why is chlorophyll b only used in light-harvesting systems? Journal of Plant Research, 131:961-972.

Lee, E. H., Lee, B. E. and Kim, J. G. (2018). Effects of water levels and soil nutrients on the growth of Iris laevigata seedlings. Journal of Ecology and Environment, 42:1-7.

Liang, Y., Urano, D., Liao, K. L., Hedrick, T. L., Gao, Y. and Jones, A. M. (2017). A nondestructive method to estimate the chlorophyll content of Arabidopsis seedlings. Plant Methods, 13:1-10.

Limantara, L., Dettling, M., Indrawati, R., Indriatmoko and Brotosudarmo, T. H. P. (2015). Analysis on the Chlorophyll Content of Commercial Green Leafy Vegetables. Procedia Chemistry, 14:225-231.

Liu, Y., Hatou, K., Aihara, T., Kurose, S., Akiyama, T., Kohno, Y., Lu, S. and Omasa, K. (2021). Assessment of naked barley leaf SPAD values using RGB values under different growth stages at both the leaf and canopy levels. Eco-Engineering, 33:31-38.

Lopez, G., Ahmadi, S. H., Amelung, W., Athmann, M., Ewert, F., Gaiser, T., Gocke, M. I., Kautz, T., Postma, J., Rachmilevitch, S., Schaaf, G., Schnepf, A., Stoschus, A., Watt, M., Yu, P. and Seidel, S. J. (2023). Nutrient deficiency effects on root architecture and root-to-shoot ratio in arable crops. Frontiers in Plant Science, 13:1-18.

Morandi, B., Losciale, P., Manfrini, L., Zibordi, M., Anconelli, S., Galli, F., Pierpaoli, E. and Corelli Grappadelli, L. (2014). Increasing water stress negatively affects pear fruit growth by reducing first its xylem and then its phloem inflow. Journal of Plant Physiology, 171:1500-1509.

Nkcukankcuka, M., Jimoh, M. O., Griesel, G. and Laubscher, C. P. (2022). Growth characteristics, chlorophyll content and nutrients uptake in *Tetragonia decumbens* Mill. Cultivated under different fertigation regimes in hydroponics. Crop and Pasture Science, 73:67-76.

Ojolo, S. P., Cao, S., Priyadarshani, S. V. G. N., Li, W., Yan, M., Aslam, M., Zhao, H. and Qin, Y. (2018). Regulation of plant growth and development: a review from a chromatin remodeling perspective. Frontiers in Plant Science, 9:1-13.

Osakabe, Y., Osakabe, K., Shinozaki, K. and Tran, L. S. P. (2014). Response of plants to water stress. Frontiers in Plant Science, 5:1-8.

Özreçberoğlu, N. and Kahramanoğlu, I. (2020). Mathematical models for the estimation of leaf chlorophyll content based on RGB colours of contact imaging with smartphones: A pomegranate example. Folia Horticulturae, 32:57-67.

Pascual, M. P., Lorenzo, G. A. and Gabriel, A. G. (2018). Vertical Farming Using Hydroponic System: Toward a Sustainable Onion Production in Nueva Ecija, Philippines. Open Journal of Ecology, 08:25-41.

Putra, G. M. D., Sutiarso, L., Nugroho, A. P. and Ngadisih. (2023). Correlation coefficient estimation of red chili (*Capsicum annuum* L.) stomatal parameters under shade treatment condition. IOP Conference Series: Earth and Environmental Science, 1182:012001.

Putra, G. M. D., Sutiarso, L., Nugroho, A. P., Ngadisih, N. and Chaer, M. S. I. (2022). Stomatal response of red chili (*Capsicum annuum* L.) due to water stress. International Journal of Agricultural Technology, 18:2545-2558.

Riccardi, M., Mele, G., Pulvento, C., Lavini, A., D'Andria, R. and Jacobsen, S. E. (2014). Non-destructive evaluation of chlorophyll content in quinoa and amaranth leaf by simple and multiple regression analysis of RGB image components. Photosynthesis Research, 120:263-272.

Saeed, M., Ilyas, N., Akram, A. and Raja, N. I. (2016). Effect of drought stress on Brassica crops and its mitigation by inoculation of PGPR. International Journal of Biosciences (IJB), 9:282-291.

Setiawati, D. A., Sutiarso, L., Ngadisih and Murtiningrum (2023a). Plant stress prediction model on nutrient film technique hydroponic in a natural greenhouse. IOP Conference Series: Earth and Environmental Science, 1182.

Setiawati, D. A., Sutiarso, L., Ngadisih, Murtiningrum, Nugroho, A. P., Putra, G. M. D. P. and Chaer, M. S. I. (2023b). Mathematic Modelling of Bok Choy Plant Canopy Area on Different Artificial Light at Plant Factory. Proceedings of the International Conference on Sustainable Environment, Agriculture and Tourism (ICOSEAT 2022), 26:3-11.

Shah, S. H., Houborg, R. and McCabe, M. F. (2017). Response of Chlorophyll, Carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). Agronomy, 7:1-21.

Shamshiri, R. and Ismail, W. I. W. (2013). A Review of greenhouse climate control and automation systems in tropical regions. Journal of Agricultural Science and Applications, 02:175-182.

Shibghatallah, M. A. H., Khotimah, S. N., Suhandono, S., Viridi, S. and Kesuma, T. (2013). Measuring leaf chlorophyll concentration from its color: A way in monitoring environment change to plantations. AIP Conference Proceedings, 1554:210-213.

Tao, M., Ma, X., Huang, X., Liu, C., Deng, R., Liang, K. and Qi, L. (2020). Smartphone-based detection of leaf color levels in rice plants. Computers and Electronics in Agriculture, 173.

Valverde, J. C. (2021). Estimation of leaf nitrogen content from non- destructive methods in Eucalyptus tereticornis and Eucalyptus saligna plantations Estimación del contenido de nitrógeno foliar por métodos. Revista Facultad Nacional de Agronomía Medellín, 74:9655-9666.

Veazie, P., Cockson, P., Henry, J., Perkins-Veazie, P. and Whipker, B. (2020). Characterization of nutrient disorders and impacts on chlorophyll and anthocyanin concentration of Brassica rapa var. Chinensis. Agriculture (Switzerland), 10:1-16.

Wang, Q., Chen, J., Stamps, R. H. and Li, Y. (2005). Correlation of visual quality grading and SPAD reading of green-leaved foliage plants. Journal of Plant Nutrition, 28:1215-1225.

Yadav, S. P., Ibaraki, Y. and Gupta, S. D. (2010). Estimation of the chlorophyll content of micropropagated potato plants using RGB based image analysis. Plant Cell, Tissue and Organ Culture, 100:183-188.

Yang, W., Lu, X., Zhang, Y. and Qiao, Y. (2019). Effect of cooking methods on the health-promoting compounds, antioxidant activity and nitrate of tatsoi (*Brassica rapa* L. ssp. narinosa). Journal of Food Processing and Preservation, 43:1-8.

Zou, L., Tan, W. K., Du, Y., Lee, H. W., Liang, X., Lei, J., Striegel, L., Weber, N., Rychlik, M. and Ong, C. N. (2021). Nutritional metabolites in *Brassica rapa* subsp. chinensis var. parachinensis (choy sum) at three different growth stages: Microgreen, seedling and adult plant. Food Chemistry, 357:129535.

(Received: 13 February 2024, Revised: 4 July 2024, Accepted: 9 July 2024)