

Determination of the Vegetative Phase Critical for Monitoring Wheat Nitrogen Status Tropical Through Digital Color Analysis and Index Camera Reflectance

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ABSTRACT

Reliable nitrogen (N) monitoring remains a major challenge in tropical wheat systems due to dynamic N uptake, redistribution, and environmental variability across growth stages. Although RGB based digital imaging offers a rapid and non-destructive alternative, its accuracy is often limited by the phase-dependent relationship between leaf color and nitrogen status. This study aimed to identify the critical vegetative stage that provides the most reliable estimation of leaf nitrogen in tropical wheat (*Triticum aestivum* L.). A greenhouse experiment was conducted using a randomized block design with five nitrogen rates (0–300 kg urea ha⁻¹). Leaf images were collected at four vegetative stages (V1–V4), and RGB values were transformed into vegetation indices (ExG, GLL, TGI, and NGRDI). These indices were correlated with chlorophyll and leaf nitrogen content using Pearson analysis. Results showed a clear phase-specific pattern, with the strongest relationship observed at V3 (second node emergence). The ExG index exhibited a very strong and significant correlation with leaf nitrogen ($r = 0.97$; $p < 0.01$). In contrast, weak correlations at V1 and negative trends at V4 were associated with limited nitrogen accumulation and remobilization processes. These findings highlight V3 as the optimal stage for nitrogen estimation and emphasize the importance of a phase-specific approach to improve RGB based monitoring in tropical wheat systems.

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1. INTRODUCTION

Nitrogen (N) is an essential macronutrient that plays a crucial role in the growth and productivity of wheat (*Triticum aestivum* L.). Nitrogen functions as a key component in the formation of chlorophyll, proteins, enzymes, and various metabolic compounds that directly influence

photosynthesis and plant growth. Adequate nitrogen availability has been shown to enhance photosynthetic capacity, biomass formation, and grain yield and quality, particularly protein content, which is a major parameter in the wheat-based food industry [4], [35]. Conversely, nitrogen imbalance, both deficiency and excess, can disrupt plant

physiological processes. Nitrogen deficiency reduces chlorophyll content and photosynthetic activity, resulting in pale or yellowish leaves, while excessive nitrogen leads to dark green leaves, excessive vegetative growth, increased disease susceptibility, and reduced yield efficiency [4], [13], [23].

In tropical wheat cultivation systems, nitrogen management becomes more complex due to environmental factors such as high temperature, solar radiation, rainfall variability, and soil moisture dynamics. These factors affect nitrogen availability in soil, uptake efficiency, and distribution within plants. Therefore, rapid, efficient, and accurate methods for monitoring plant nitrogen status are required. One of the most commonly used indicators is leaf color, as nitrogen is a key component of chlorophyll that determines leaf greenness. Plants with sufficient nitrogen typically exhibit green leaves, whereas nitrogen deficiency leads to reduced greenness due to chlorophyll degradation [4].

Various methods have been developed to assess plant nitrogen status. Destructive methods such as the Kjeldahl technique provide accurate results but are time-consuming and require hazardous chemicals [10]. Non-destructive methods such as SPAD and Leaf Color Chart (LCC) are more practical but have limitations in accuracy and sensitivity to environmental and morphological factors. SPAD measures relative chlorophyll content, while LCC relies on visual perception and lighting conditions during measurement [27]. With technological advancements, RGB-based digital image analysis has emerged as a promising alternative for non-destructive nitrogen monitoring.

Digital cameras capture spectral information through red (R), green (G), and blue (B) channels that are closely related to plant physiological conditions. Vegetation indices derived from RGB channels, such as Excess Green (ExG), Triangular Greenness Index (TGI), Green Leaf Index (GLI), and Normalized Green-Red Difference Index

(NGRDI), are widely used to assess leaf greenness and indirectly estimate chlorophyll and nitrogen content. These approaches offer advantages in terms of efficiency, cost, and integration with precision agriculture technologies [8], [30]. RGB-based indices, particularly those emphasizing the green channel, have shown strong correlations with chlorophyll and nitrogen content [17], [29].

However, the relationship between leaf color and nitrogen status is not straightforward because it is influenced by plant physiological dynamics. Nitrogen is a mobile element that can be translocated from older tissues to younger growing tissues. Under nitrogen deficiency, remobilization causes chlorosis in older leaves while younger leaves remain green [13]. Furthermore, nitrogen distribution changes throughout plant growth stages, with accumulation peaking during the vegetative phase and subsequently being redistributed to reproductive organs [16]. This indicates that leaf nitrogen content at a given stage does not always represent the overall plant nitrogen status.

Wheat growth consists of sequential stages, from germination to reproductive development, including tillering, stem elongation, and flowering [GRDC]. During vegetative stages, particularly from tillering to flag leaf emergence, nitrogen uptake and accumulation are highly dynamic. Early vegetative stages are characterized by limited nitrogen uptake due to underdeveloped root systems, while active vegetative stages show increased nitrogen accumulation. In later stages, nitrogen begins to be remobilized to support reproductive development.

These variations indicate that the accuracy of nitrogen estimation based on leaf color or digital imaging is highly dependent on the plant growth stage. Differences in nitrogen dynamics across vegetative phases lead to inconsistent relationships between color indices and nitrogen content. Therefore, identifying the most representative vegetative stage is essential to improve the accuracy of nitrogen monitoring models.

To date, studies specifically identifying the critical vegetative stage for nitrogen monitoring in tropical wheat using digital image analysis remain limited. Consequently, there is no clear consensus on which stage best represents plant nitrogen status. This uncertainty may introduce bias in nitrogen estimation if measurements are conducted at inappropriate growth stages. Therefore, this study aims to determine the most accurate vegetative phase for estimating leaf nitrogen status using RGB-based digital imaging and vegetation indices in tropical wheat (Selayar variety).

2. LITERATURE REVIEW

2.1 *Wheat Plants*

Wheat (*Triticum aestivum* L.) is an allohexaploid plant belonging to the Poaceae family and originating from subtropical regions [5], [19]. Wheat growth occurs through several sequential phases, including germination, tillering, stem elongation, flowering, and grain filling until maturity [GRDC]. The flag leaf plays a critical role in supporting photosynthesis during grain filling, contributing significantly to final yield.

Under tropical conditions, wheat requires specific environmental conditions, including optimal temperatures of 12–23°C, annual rainfall of 350–1,250 mm, and soil pH ranging from 6.0 to 8.2 with good drainage [28]. The availability of macronutrients, especially nitrogen, phosphorus, and potassium, is essential for optimal plant growth. Proper fertilization management during early and late vegetative stages is necessary to enhance crop productivity [25].

Tropical wheat varieties such as Selayar exhibit strong agronomic performance,

including high tiller numbers, large grain weight, and high productivity [26], [7]. These varieties also show good adaptation to high temperatures and stable reproductive performance [24]. Additionally, their high carbohydrate and protein content makes them suitable for food industry applications [2].

2.2 *Nitrogen Nutrients in Wheat Plants*

Nitrogen is an essential macronutrient that plays a key role in plant growth, biomass formation, and grain yield in wheat [18], [35]. Nitrogen is absorbed in the form of nitrate and ammonium and distributed to various plant organs, particularly leaves and stems. It is involved in the synthesis of chlorophyll, proteins, and other metabolic compounds that regulate photosynthesis and plant development [4].

Adequate nitrogen availability results in optimal plant growth, characterized by green leaves, well-developed root systems, and efficient grain formation [18], [16]. In contrast, nitrogen deficiency leads to reduced plant height, leaf area, and biomass, along with chlorosis in older leaves due to reduced chlorophyll content [35], [13]. This condition also affects photosynthetic efficiency and triggers physiological stress responses, including increased stress hormones [1], [3].

Excess nitrogen causes excessive vegetative growth, characterized by dark green leaves and increased plant height [14]. This condition increases the risk of lodging and reduces resource use efficiency.

Furthermore, excessive nitrogen disrupts biomass allocation between vegetative and reproductive organs, ultimately reducing grain yield [34], [23].

2.3 *Plant Nitrogen Status Prediction Techniques*

2.3.1 SPAD

SPAD is a non-destructive method used to estimate leaf chlorophyll content based on red and infrared light absorbance [20], [12]. SPAD values are strongly correlated with plant nitrogen content and can be used as an indicator of nitrogen status across growth stages [36].

SPAD measurements have also been shown to correlate positively with wheat grain yield, particularly during the reproductive phase [21]. However, SPAD readings are influenced by environmental conditions, plant varieties, and leaf characteristics such as thickness, requiring proper calibration for accurate interpretation [6].

Leaf anatomical structure also affects light distribution within leaf tissues, influencing SPAD readings [32]. Therefore, although SPAD is practical and rapid, its accuracy depends on physiological and environmental factors.

2.3.2 Kjeldahl

The Kjeldahl method is a standard technique for determining total nitrogen content through digestion, distillation, and titration processes [10]. Organic nitrogen is converted into ammonium sulfate during digestion, followed by ammonia distillation and quantification through titration.

This method provides high accuracy and is widely used as a reference for nitrogen analysis. However, it requires a long processing time and involves hazardous chemicals, making it less suitable for field applications.

2.3.3 Leaf Color Chart (LCC)

The Leaf Color Chart (LCC) is a simple visual tool used to estimate nitrogen status based on leaf greenness [31]. It consists of a series of green color panels used as references for comparing leaf color directly in the field.

LCC has been shown to improve nitrogen fertilizer efficiency and support decision-making in fertilization practices [15]. It is widely used due to its simplicity and low cost.

However, LCC has limitations in sensitivity and accuracy because it depends on visual perception and environmental conditions during measurement [27]. Additionally, variations in plant variety and other nutrient deficiencies may influence the results.

2.4 *Utilization of RGB Values for Nitrogen Prediction*

Digital imaging systems capture light using sensors that convert light energy into electrical signals through pixels sensitive to red, green, and blue wavelengths [11]. Each pixel represents color information that forms the basis for digital image analysis.

The RGB color model represents color as a combination of three channels, enabling the representation of millions of colors [9]. This model is widely used in plant image

analysis to evaluate physiological conditions.

RGB-based image analysis allows estimation of plant nitrogen status by analyzing leaf color changes, particularly in the green channel [8], [30]. Vegetation indices derived from RGB data, such as ExG and NGRDI, can detect early nitrogen deficiency symptoms [37], [29]. These methods can be further enhanced using machine learning approaches to improve prediction accuracy [17].

3. METHODS

3.1 Time, Location and Design Study

Study held from September to November 2025 at home glass, garden experiments and laboratories land Faculty Agriculture and Business, Satya Wacana Christian University, Salatiga, Central Java. study is experiment quantitative with Randomized Block Design (RBD), five levels treatment, namely 0 kg (N0), 75 kg (N1), 150 kg (N2), 225 kg (N3), and 300 kg (N4) Urea ha^{-1} . Each treatment was repeated 5 times, and varieties wheat used is Selayar . Wheat planted in planter bag with soil media from Andosol, where every planter bag planted 30 plant wheat.

3.2 Digital Image Capture of Leaves

Digital image of a leaf taken on the leaves mature from every treatment, on four phase vegetative, namely phase formation offspring (V1) around 21 days to maturity (DTM), elongation stem (V2) about 28 DTM, emergence book second (V3) about 42 DTM, and emergence leaf flag (V4) around 48 DTM. In each experimental unit, selected a number of healthy and representative leaves. Taking digital image of a leaf carried out under conditions lighting relatively natural uniform in the middle day around 12.00-13.00 for minimize variation intensity light. The leaves are placed on a background behind dark for

increase contrast object to background, so that simplify the segmentation process image. The distance between the camera and object (leaf) are guarded constant that is 30 cm, and corner taking arranged for minimize effect reflection light on the surface leaf. Digital image of the leaf obtained saved in digital format for analyzed more carry on.

3.3 Leaf Digital Image Analysis (Analysis Index Vegetation)

Analysis digital color from digital image of a leaf done through device soft processing image with stages segmentation object leaf from background behind use color threshold approach. Selected leaf area Then used as a region of interest (ROI) for extracting mark intensity color on the channel red (R), green (G), and blue (B). For reduce influence variation lighting, RGB values are converted become form normalized (r, g, b). Next, some index vegetation-based color calculated, including Excess Green (ExG), Triangular Greenness Index (TGI), Green Leaf Index (GLI), and Normalized Green-Red Difference Index (NGRDI). These indices This used as indicator No direct from content chlorophyll and leaf nitrogen. The equation used is as following:

- Normalization of RGB data from digital image of a leaf use equality
 - $r = R/(R + G + B)$ (1)
 - $g = G/(R + G + B)$ (2)
 - $b = B/(R + G + B)$ (3)
- Index vegetation use equality:
 - $\text{ExG} = 2g - r - b$ (4)
 - $\text{TGI} = -0.5 \times [190(R - G) - 120(R - B)]$ (5)
 - $\text{GLI} = (2G - R - B)/(2G + R + B)$ (6)
 - $\text{NGRDI} = (G - R)/(G + R)$ (7)

Next, all data is normalized. For equalize scale between variables and reduce analysis bias.

3.4 Measurement Leaf Chlorophyll and Leaf Tissue N Analysis

Chlorophyll a, b and total leaf measured with method dimethyl sulfoxide (DMSO) extraction without grinding process, using spectrophotometer. Analysis of N tissue leaf measured with method destruction wet Then distilled in condition language for release ammonia, which is then arrested in solution container and titrated use solution standard. Total nitrogen content is calculated based on the volume of titrant used.

3.5 Analysis Statistics

Index data vegetation obtained analyzed statistics with device IBM SPSS

software version 26.0 Analysis Pearson correlation is used for look for connection between index digital color with content chlorophyll and tissue nitrogen analysis Pearson correlation was performed on each data from every phase vegetative for identify phase growth that has strongest relationship between color parameters and plant nitrogen status. The coefficient value correlation used as base for determine index color and phase the most representative vegetative in monitoring nitrogen status in plants wheat tropical. As for the stages study in a way overall can seen in Figure 1.

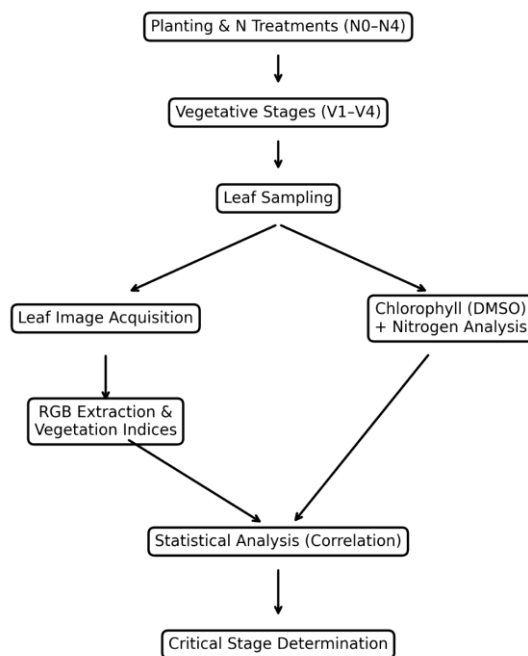






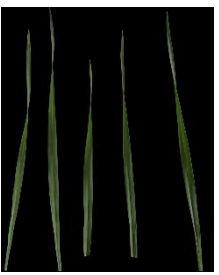
Figure 1. Flowchart Study for Determine Phase Vegetative Critical in Monitoring Plant Nitrogen Wheat Based Digital Image.





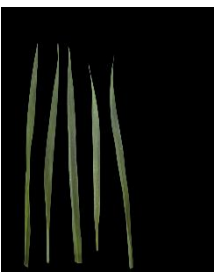
4. RESULTS AND DISCUSSION





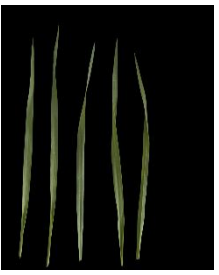
4.1 Visual differences in color leaf wheat and RGB value on various level nitrogen application (N0-N4)

Has occurred difference color leaf from low N dose namely N0 (without nitrogen) to to high N namely N4 with color green dark in various phase plant wheat (Figure 2). Conditions This happen Because existence improvement content chlorophyll

stimulated by increased nitrogen. Indications change color seen in a way quantitative in changing RGB values in a way significant, where N application improves G (Green) and chlorophyll values absorb light red/blue and reflect light green.

Phase 1 (V1)				
				
(N0) average value $\bar{x}R=7.40$ $\bar{x}G=8.66$ $\bar{x}B=5.05$	(N1) $\bar{x}R= 8.45$ $\bar{x}G= 9.62$ $\bar{x}B= 5.14$	(N2) $\bar{x}R= 8.84$ $\bar{x}G = 10.54$ $\bar{x}B = 5.28$	(N3) $\bar{x}R = 9.06$ $\bar{x}G = 9.98$ $\bar{x}B = 5.78$	(N4) $\bar{x}R = 8.16$ $\bar{x}G = 9.83$ $\bar{x}B = 5.72$

Phase 2 (V2)				
				
(N0) $\bar{x}R = 9.15$ $\bar{x}G = 10.339$ $\bar{x}B = 6.70$	(N1) $\bar{x}R = 8.95$ $\bar{x}G = 10.22$ $\bar{x}B = 6.60$	(N2) $\bar{x}R = 9.39$ $\bar{x}G = 10.72$ $\bar{x}B = 6.60$	(N3) $\bar{x}R = 9.68$ $\bar{x}G = 10.95$ $\bar{x}B = 6.95$	N4 $\bar{x}R = 10.28$ $\bar{x}G = 11.75$ $\bar{x}B = 7.07$

Phase 3 (V3)				
				
(N0) $\bar{x}R = 7.91$	(N1) $\bar{x}R = 8.86$	(N2) $\bar{x}R = 9.37$	(N3) $\bar{x}R = 8.88$	N4 $\bar{x}R = 9.89$


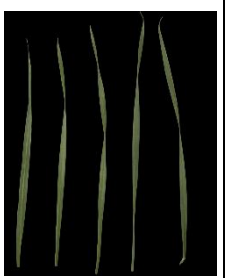



$\bar{x}G = 8.63$ $\bar{x}B = 5.38$	$\bar{x}G = 10.06$ $\bar{x}B = 6.21$	$\bar{x}G = 10.30$ $\bar{x}B = 6.36$	$\bar{x}G = 10.15$ $\bar{x}B = 6.38$	$\bar{x}G = 11.18$ $\bar{x}B = 6.59$
Phase 4 (V4)				
				
(N0) $\bar{x}R = 8.80$ $\bar{x}G = 10.07$ $\bar{x}B = 6.51$	(N1) $\bar{x}R = 8.87$ $\bar{x}G = 9.97$ $\bar{x}B = 6.31$	(N2) $\bar{x}R = 9.53$ $\bar{x}G = 10.66$ $\bar{x}B = 6.84$	(N3) $\bar{x}R = 7.78$ $\bar{x}G = 8.75$ $\bar{x}B = 5.71$	N4 $\bar{x} = 7.97$ $\bar{x}G = 9.10$ $\bar{x}B = 5.94$

Figure 2. Visual differences in wheat leaf color under varying nitrogen application rates (N0–N4). Increasing nitrogen levels result in progressively darker green coloration and higher Green (G) on RGB, indicating higher chlorophyll and nitrogen content

Source: Processed Primary Data (2026)

Figure 2 shows that increase nitrogen dose produces color the leaves are getting green old and improved mark channel green (G) in RGB, which reflects improvement content chlorophyll and nitrogen in leaves. In quantitative pattern improvement mark channel green (G) visible more clear in phases V2 and V3 compared phase others. In the V2 phase, the G value increases from 10.33 (N0) to 11.75 (N4), while in phase V3 it increased from 8.63 (N0) to 11.18 (N4). Increase This show that in the phase vegetative active, responsive color leaf to nitrogen to become more sensitive and consistent. In contrast, in the V1 phase the increase RGB values still relatively small and not consistent, whereas in phase V4 there is decline RGB values on some treatment, which shows that connection between color leaves and nitrogen start weakening. Increase intensity color green on the leaves related close with improvement synthesis chlorophyll consequence greater nitrogen availability high. Nitrogen is component main in formation chlorophyll, so

that increase in nitrogen will increase capacity photosynthesis and intensity greenish leaves [4], [35]. Therefore that, the channel green (G) in digital images become the most sensitive indicator in detect changes in plant nitrogen status.

However, response color leaf against nitrogen no nature constant throughout phase growth. In the growth phase initial (V1), system roots Not yet develop optimally so that nitrogen uptake is still limited, causing difference color leaf between treatment Not yet seen clear. On the other hand, in phases V2 and V3, the plants is in phase vegetative active with high nitrogen accumulation, so that difference color leaf between nitrogen dose becomes more real. Meanwhile that, in phase V4, the decrease consistency RGB values indicate that color leaf No Again in a way direct represent nitrogen content due to change nitrogen distribution in plants, especially the remobilization process towards the reproductive organs [35].

4.2 Influence nitrogen dose to content chlorophyll and tissue nitrogen leafon every phase vegetative

Tissue nitrogen content leaf increase along with improvement dose nitrogen

fertilization from N0 to N4 in various phase vegetative plant wheat (Figure 3), which shows response positive plant to improvement nitrogen availability through improvement absorption and accumulation in network leaf.

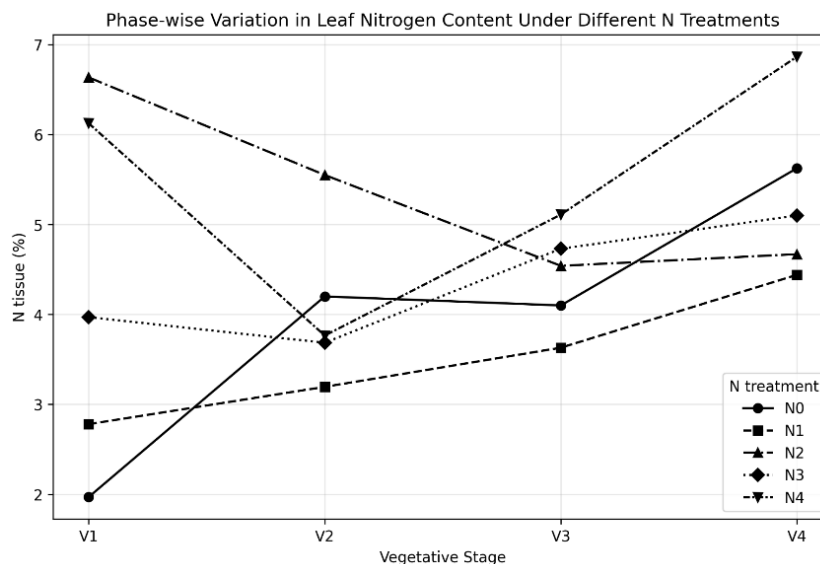


Figure 3. Graph of Phase-Wise Variation in Leaf Nitrogen Content Under Different Nitrogen Treatments

The graph in Figure 3 shows that tissue nitrogen content leaf varies between level fertilization and phase growth, with trend increased at higher nitrogen doses high, even though No always linear throughout phase. In phase V1, the nitrogen content is still relatively low and difference between treatment Not yet clear, which shows that nitrogen uptake is still limited consequence development system rooting is not optimal. Entering phases V2 and V3, the nitrogen content increases in a way more consistency and difference between level fertilization become more firm, reflective improvement nitrogen accumulation during phase vegetative active.

This pattern show that nitrogen dynamics in plants are greatly influenced by the phase growth, where phase vegetative active is period the most intensive accumulation of nitrogen. This is in line with Wang et al. [35] which states that nitrogen plays a role important in formation biomass and activity photosynthesis, as well as accumulated optimally in the phase growth

vegetative. In addition, studies by Li et al. [16] shows that nitrogen accumulation in leaf increase along with activity metabolism plants and become more stable in phase vegetative active.

However, in the V4 phase the pattern nitrogen content becomes not enough consistent, especially in some treatment dose high. This indicates occurrence remobilization of nitrogen from leaf to the reproductive organs, so leaf nitrogen content No Again reflects the total available nitrogen in plants [35]. Conditions This confirm that connection between leaf nitrogen and factors physiological other nature dynamic and changing throughout cycle growth.

4.2.1 Chlorophyll leaf

Improvement dose nitrogen fertilization from N0 to N4 followed by an increase content chlorophyll leaf on various phase vegetative plant wheat (Figure 4). This show that nitrogen availability plays a role important in push synthesis chlorophyll, so

that leaf become more green and reflective improvement activity photosynthesis.

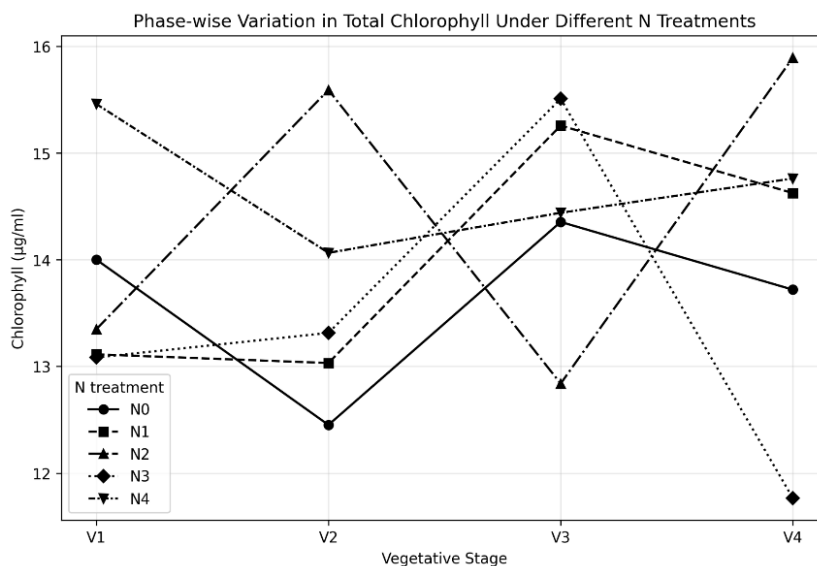


Figure 4. Graph of Phase-Wise Variation in Total Chlorophyll Under Different Nitrogen Treatments.

The graph in Figure 4 shows that content chlorophyll leaf varies between level nitrogen fertilization and phase growth plants. In general, value chlorophyll tend increased with more nitrogen treatment high, especially in phases V2 and V3, which indicates that nitrogen availability plays a role important in support synthesis chlorophyll during phase vegetative active. In the V1 phase, the difference between treatment Still Not yet clear, which indicates that accumulation chlorophyll not optimal in the phase beginning growth.

Improvement content more chlorophyll real in phase V2 and V3 shows that connection between nitrogen and chlorophyll become more stable along increasing activity metabolism plants. This is in line with Li et al. [16] who reported that content chlorophyll own deeper relationship strong with nitrogen in the phase vegetative active. In addition, nitrogen is component main in molecule chlorophyll, so that improvement nitrogen availability will direct increase capacity photosynthesis and intensity greenish leaves [4].

However, in the V4 phase the pattern content chlorophyll become not enough consistent between treatment, although tissue nitrogen in some treatment Still relatively high. This is show that connection between chlorophyll and nitrogen do not Again linear in phase continue. In physiological, condition This related with the process of nitrogen remobilization to the reproductive organs, while chlorophyll in leaves still maintained for support activity photosynthesis during filling seeds [35].

4.3 Relationship between color parameters (RGB) with N network content leaf

Connection between color parameters leaf based RGB channels with content total chlorophyll shows variation between phase vegetative plant wheat (Table 1). This pattern indicates that ability color leaf in represent condition physiological plant No nature constant, but rather influenced by the phase growth.

Table 1. Correlation RGB channels with total chlorophyll in each leaf phase vegetative

Phase	Green- Total chlorophyll	Red- Total chlorophyll	Blue- Total chlorophyll
V1	0.58 (p = 0.305)	0.45 (p = 0.447)	0.15 (p = 0.810)
V2	0.04 (p = 0.949)	-0.04 (p = 0.949)	-0.27 (p = 0.660)
V3	0.83 (p = 0.082)	0.80 (p = 0.104)	0.75 (p = 0.144)
V4	-0.17 (p = 0.785)	-0.23 (p = 0.710)	-0.34 (p = 0.576)

Source: Processed Primary Data (2026)

Description: Correlation value counted based on data on each phase vegetative using five levels nitrogen treatment (N0, N1, N2, N3, and N4), with tissue nitrogen leaf as variables response. Analysis done in a way partial per phase, so that every mark correlation represent connection between color and chlorophyll parameters on average all over condition nitrogen treatment in same phase.

Based on Table 1, the relationship between RGB channels and total leaf chlorophyll different between phase vegetative. Correlation highest found in phase V3, especially in the canal green ($r = 0.83$), red ($r = 0.80$), and blue ($r = 0.75$), which indicate that in the phase This color the most capable leaf reflect content chlorophyll. However, all over connection the Still Not yet significant in a way statistics ($p > 0.05$), which indicates that use raw RGB channels Still own limitations in detect chlorophyll leaf in a way consistent. In contrast, in phases V2 and V4, the correlation tends weak, showing that connection between color leaves and chlorophyll are greatly influenced by the phase growth.

Limitations the show that raw RGB values Not yet capable catch variation physiological leaf in a way specific, because it is greatly influenced by factors external like intensity light and conditions surface leaves. Therefore, that required transformation RGB values become index more vegetation sensitive to component greenish leaf. Index vegetation RGB-based such as ExG, GLI, TGI, and NGRDI are capable of extracting information color in a way more specific and reduce influence variation environment.

4.4 Relationship index vegetation RGB based with leaf nitrogen content

Connection between index vegetation RGB-based and leaf nitrogen content show different patterns on each phase vegetative plant wheat (Table 2). This pattern indicates that ability index vegetation in represents the nitrogen status of nature dynamic and highly influenced by the phase growth plant.

Table 2. Correlation and p-value between index RGB vegetation and leaf nitrogen content in various phase vegetative

Vegetative phase	ExG -N Network (%)	Network GLI-N (%)	NGRDI-N Network (%)	TGI-N Network (%)
V1	-0.33 (ns)	-0.63 (ns)	0.34 (ns)	-0.21 (ns)
V2	0.74 (ns)	0.54 (ns)	0.30 (ns)	0.59 (ns)
V3	0.97 (**)	0.28 (ns)	0.29 (ns)	0.55 (ns)
V4	-0.77 (ns)	-0.79 (ns)	-0.80 (ns)	-0.40 (ns)

Source: Processed Primary Data (2026)

Description: Correlation value counted based on data on each phase vegetative using five levels nitrogen treatment (N0, N1, N2, N3, and N4), with tissue nitrogen content leaves (%) as variables response. Index vegetation (ExG, GLI, NGRDI, and TGI) were calculated from RGB values of the image leaves in each treatment, and analysis correlation done in a way partial per phase, so that every mark represent connection between index vegetation and nitrogen under conditions nitrogen treatment in same phase.

The results of the analysis show that connection between index vegetation RGB-based and leaf nitrogen content is greatly influenced by the phase growth plants. In the phase beginning (V1), all index show correlation weak until moderate and dominated by relationships negative. This show that in the phase beginning vegetative, response spectral leaf Not yet capable represents the nitrogen status in accurate, related with Still limited nitrogen absorption and distribution of nitrogen that has not been stable in network plants [13], [4].

Entering phase elongation stem (V2), whole index show correlation positive, with

mark highest in ExG ($r = 0.74$). The increase indicates that connection between color leaves and nitrogen content begins to formed more consistent along increasing activity physiological plants. In the phase this, nitrogen plays a role important in synthesis chlorophyll and formation biomass, so that change nitrogen content starts reflected in intensity greenish leaves [16].

Connection strongest found in the phase emergence book second (V3), where index ExG show very strong and significant correlation ($r = 0.97$; $p < 0.01$). This value show that in the phase said, index based channel green capable represent leaf nitrogen content optimally. In physiologically, phase V3 is period peak activity vegetative, where nitrogen accumulation in leaf reach condition maximum and contribute direct to synthesis chlorophyll [16], [35].

For clarify dynamics connection between index vegetation and leaf nitrogen content between phase growth, presented variation coefficient correlation index ExG against nitrogen in Figure 5. Graph the show that mark correlation increases from phase V1 to reach peak in phase V3, then decrease in a way sharp in the V4 phase.

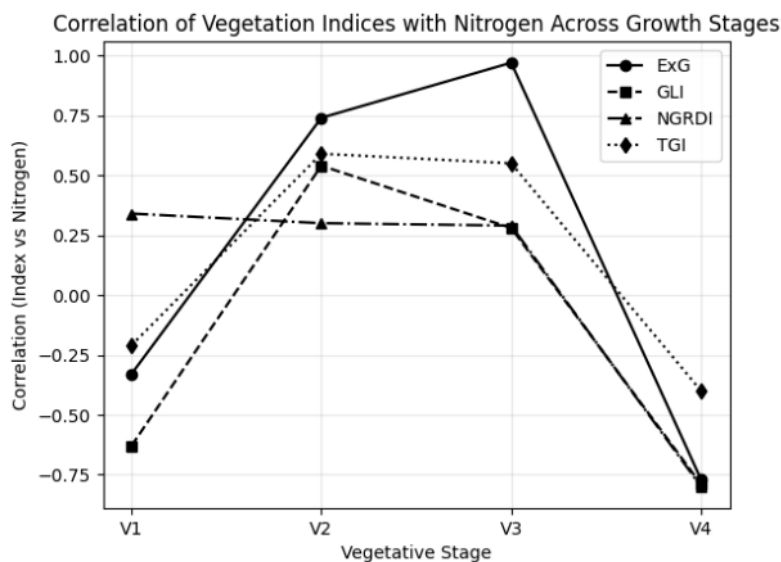


Figure 5. Variation in the correlation coefficient between the ExG index and leaf nitrogen content across vegetative growth stages. The correlation increases from V1 to a peak at V3, followed by a sharp decline at V4

In contrast, in the V4 phase all index show correlation strong negative. Changes direction correlation show that index vegetation No Again represent nitrogen

content in general straight to the phase continue. Condition This related with the process of nitrogen remobilization from leaf to the reproductive organs during phase

development generative [35]. In addition, the senescence process leaves also participate influence connection between color leaves and nitrogen content in phase further.

The relationship between RGB-based vegetation indices and nitrogen content is phase-specific and non-linear throughout the plant growth cycle. Findings This confirm that election phase proper vegetative is factor key in increase nitrogen-based monitoring accuracy digital image, where V3 phase identified as the most critical and representative phase.

The pattern shows that sensitivity index ExG to nitrogen is highly dependent on the phase growth plants. Significant improvement in V2 to phase reach mark maximum in phase V3 reflects condition physiological the most optimal plants, where nitrogen accumulation and synthesis chlorophyll be at the level high and relative homogeneous [16], [35]. On the other hand, the decrease in phase V4 indicates that connection between color leaves and nitrogen do not Again consistent, related with the process of nitrogen remobilization to reproductive organs [35].

Findings This confirm that nitrogen monitoring based on digital images require approach-based phase (phase-specific approach), because accuracy estimates are highly dependent on dynamics physiological plant. Phase V3 identified as the most optimal conditions, where connection between index vegetation and nitrogen reach consistency maximum.

5. CONCLUSION

Study results show existence connection close (correlation) between index

vegetation RGB based with plant nitrogen and total chlorophyll, will but correlation the determined factor phase grow plants. Therefore, That results study open opportunity development nitrogen- based monitoring system more digital images adaptive to dynamics phase growth plant wheat tropical.

Based on N network data analysis plants, chlorophyll leaves, and RGB index on various phase grow plants, then For wheat varieties Selayar show phase vegetative stage 3 (V3) namely moment plant 42- day wheat after plant seeds, and on the stem Already found book second *node* as the most optimal conditions for do assessment of plant N status with index vegetation ExG, where in the phase the index vegetation ExG capable show correlation highest and most significant to total leaf nitrogen and chlorophyll content.

In phase initial (V1) and phase continued (V4), relationship between vegetation index RGB based with N plants tend weak and not consistent consequence limitations accumulation as well as nitrogen remobilization in network plants. These results show that accuracy nitrogen- based estimation digital images depend on suitability phase growth, so that approach-based phase (phase *specific approach*) becomes key in increase reliability of nitrogen monitoring.

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REFERENCES

- [1] T. Antal *et al.*, "Acclimation of photosynthesis to nitrogen deficiency in *Phaseolus vulgaris*," *Planta*, vol. 232, pp. 887–898, 2010.
- [2] F. Azima, N. Novelina, and R. S. Pane, "The physical and chemical properties of wheat flour in some wheat (*Triticum spp.*) varieties grown in West Sumatra," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 3, no. 4, pp. 303–308, 2013.
- [3] S. Cai *et al.*, "Evolutionary conservation of ABA signaling for stomatal closure," *Plant Physiol.*, vol. 174, no. 2, pp. 732–747, 2017.
- [4] L. H. Chen, M. Xu, Z. Cheng, and L. T. Yang, "Effects of nitrogen deficiency on photosynthesis, chlorophyll fluorescence, and antioxidant system in rice," *Int. J. Mol. Sci.*, vol. 25, no. 19, p. 10409, 2024.
- [5] F. Diekmann, "Wheat," *J. Agric. Food Inf.*, vol. 10, no. 4, pp. 289–299, 2009.
- [6] M. Esfahani *et al.*, "Improvement of nitrogen management in rice paddy fields using chlorophyll meter (SPAD)," *Paddy Water Environ.*, vol. 6, pp. 181–188, 2008.
- [7] B. M. Farid *et al.*, "Evaluation of several tropical wheat genotypes under various water availability," *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 486, p. 012093, 2020.
- [8] Y. Fu *et al.*, "Winter wheat nitrogen status estimation using UAV-based RGB imagery," *Remote Sens.*, vol. 12, no. 22, p. 3778, 2020.
- [9] R. C. Gonzalez and R. E. Woods, *Digital Image Processing*, 2nd ed. Pearson, 2002.
- [10] D. A. Goulding, P. F. Fox, and J. A. O'Mahony, "Milk proteins: An overview," in *Milk Proteins*, Academic Press, 2020, pp. 21–98.
- [11] B. K. Gunturk *et al.*, "Demosaicking: Color filter array interpolation," *IEEE Signal Process. Mag.*, vol. 22, no. 1, pp. 44–54, 2005.
- [12] T. Haider *et al.*, "A computer-vision-based approach for nitrogen content estimation in plant leaves," *Agriculture*, vol. 11, no. 8, p. 766, 2021.
- [13] J. Kang *et al.*, "Physiological mechanisms underlying reduced photosynthesis in wheat under nitrogen deficiency," *Crop J.*, vol. 11, no. 2, pp. 638–650, 2023.
- [14] L. Kong *et al.*, "Excessive nitrogen application dampens antioxidant capacity and grain filling in wheat," *Sci. Rep.*, vol. 7, p. 43363, 2017.
- [15] S. J. Leghari *et al.*, "Modern leaf color chart successfully prepared and used in crop production," *Eur. Acad. Res.*, vol. 4, no. 2, pp. 900–916, 2016.
- [16] C. Li *et al.*, "Morphology and nitrogen uptake of wheat influenced by urea application," *Agronomy*, vol. 12, no. 10, p. 2338, 2022.
- [17] R. Li *et al.*, "Estimation of nitrogen content in wheat using RGB imaging," *Field Crops Res.*, vol. 289, p. 108735, 2022.
- [18] G. Ma *et al.*, "Determining optimal nitrogen input for wheat yield," *Front. Plant Sci.*, vol. 10, p. 181, 2019.
- [19] R. A. McIntosh, "Wheat genetics," in *Reference Module in Food Science*, Elsevier, 2016.
- [20] Minolta Camera Co., Ltd., *Manual for chlorophyll meter SPAD-502*, 1989.
- [21] I. Monostori *et al.*, "Relationship between SPAD value and grain yield in wheat," *Euphytica*, vol. 211, pp. 103–112, 2016.
- [22] H. Noor and S. Min, "Effect of nitrogen rates on wheat yield and quality," *Appl. Ecol. Environ. Res.*, vol. 18, no. 4, pp. 5701–5726, 2020.
- [23] H. Noor *et al.*, "Nitrogen fertilization effects on wheat," *Heliyon*, vol. 9, no. 7, p. e18177, 2023.
- [24] A. Nur *et al.*, "Genetic improvement of tropical wheat," *J. Agric. Res. Dev.*, vol. 34, no. 1, 2015.
- [25] A. Nur *et al.*, "Genetic diversity of wheat population," *Sci. J. Isotope Radiat. Appl.*, vol. 10, no. 1, 2016.
- [26] N. E. Putri *et al.*, "Wheat adaptation in tropical regions," *SABRAO J. Breed. Genet.*, vol. 52, no. 4, 2020.
- [27] K. G. Rao and N. K. Das, "Leaf color chart for nitrogen management," *Pharma Innov. J.*, vol. 12, no. 6, pp. 3974–3979, 2023.
- [28] S. Ritung *et al.*, *Technical Guidelines for Land Evaluation for Agricultural Commodities*, 2011.
- [29] L. Rosen, P. M. Ewing, and B. C. Runck, "RGB-based indices for nitrogen estimation," *Agron. J.*, vol. 116, no. 6, pp. 3070–3080, 2024.
- [30] P. Shi *et al.*, "Nitrogen estimation using RGB images and machine learning," *Comput. Electron. Agric.*, vol. 180, p. 105860, 2021.
- [31] B. Singh, "Leaf color chart for nitrogen management," *Agric. Res. J.*, vol. 59, no. 4, 2022.
- [32] L. Taiz and E. Zeiger, *Plant Physiology*. Benjamin/Cummings, 1991.
- [33] Y. Wahyu *et al.*, "Adaptability of wheat genotypes in lowlands," *Agrohorti Bull.*, vol. 1, no. 1, pp. 1–6, 2013.

- [34] D. Wang *et al.*, "Excessive nitrogen application effects in wheat," *Acta Agric. Scand.*, 2011.
- [35] J. Wang *et al.*, "Wheat response to low nitrogen stress," *Plants*, vol. 8, no. 4, p. 98, 2019.
- [36] X. Yue *et al.*, "SPAD for nitrogen status estimation," *Int. J. Plant Prod.*, vol. 14, no. 1, pp. 67–75, 2020.
- [37] H. Yuzhu *et al.*, "Nitrogen determination using RGB image analysis," *Afr. J. Biotechnol.*, vol. 10, no. 77, pp. 17737–17741, 2011.
- [38] M. X. Zhao *et al.*, "Nitrogen accumulation in wheat," *J. Plant Nutr. Fertil. Sci.*, vol. 12, no. 2, pp. 143–149, 2006.

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