64. Contribution of visible imagery to the APPI-N fertilization method for monitoring rapeseed

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Abstract

The nitrogen nutrition index (NNI) is central to monitoring and managing the nitrogen status of crops. It can be estimated using a sampling of the aboveground biomass or optical devices like SPAD chlorophyll meter, though these are impractical for large areas. A dark green colour index (DGCI), derived from visible images was calculated and refined into a normalized nDGCI, thereby removing the need for light calibration. Trials conducted in 2023 and 2024 on rapeseed crops revealed a linear relationship (R^2=0.64) between NNI and nDGCI at the E–F1 growth stage (BBCH scale 57–60). Conversely, no linear relationship was found between normalized SPAD values (nSPAD) either NNI or nDGCI.

Keywords: DGCI, nitrogen fertilization, NNI, RGB image, SPAD

Introduction

Compared to the balance sheet method (Comifer, 2011) widely used in France, the APPI-N method represents an innovative approach for dynamic fertilization management to adjust N fertilizer amount to plant needs (Jeuffroy, 2019; Paut *et al.*, 2024; Ravier *et al.*, 2018). The concept is based on real-time monitoring of crop nitrogen status from the end of winter until flowering to optimize the nitrogen management. This monitoring relies on a key indicator: the nitrogen nutrition index (NNI), defined as the ratio of the observed nitrogen concentration in the plant aerial parts (%Nobs) to the critical nitrogen concentration (%Nc) (Lemaire *et al.*, 1989). The current reference method for measuring total nitrogen content in plants involves sampling the aerial parts of the plant. Although determining NNI through biomass is highly reliable, the method is also destructive, time-intensive, and laborious. Consequently, alternative tools have been explored to facilitate easier and faster determination of NNI for crops. One such approach uses chlorophyll content as a proxy for NNI (Confalonieri *et al.*, 2015), enabling the application of remote sensing tools for indirect assessments (Jiang *et al.*, 2021). This method relies on chlorophyll meters, such as the SPAD-502-plus (Konica Minolta, Osaka, Japan) or the N-Tester clip (Yara, France), which measure the transmittance of red and infrared light through a pinched plant leaf. The measured transmittance is directly linked to the leaf chlorophyll content, which serves as an indicator of nitrogen concentration. However, the use of these non-destructive contact infrared devices is impractical for daily and large-scale measurements. To overcome their limitations and operate at the canopy scale, the use of visible imagery using smartphones has been demonstrated in rice or maize crops at leaf scale. This technique relies on a specific colorimetric index known as the dark green color index (DGCI) although it requires a colour calibration chart (Karcher and Richardson, 2003; Rorie *et al.*, 2011). More recently, to extend the method at the canopy scale, Gée *et al.* (2023) have demonstrated the potential of an alternative index to the traditional DGCI on wheat crops: a normalized dark green colour index (nDGCI), correcting for variations in lighting and camera parameters without any calibration. The normalization involves correcting the DGCI values of the plot image with those from an over-fertilized plot. This nDGCI index can be a substitute for chlorophyll meter measurements under certain conditions. In winter wheat (*Triticum aestivum* L.) cv. LG Absalon, a linear relationship between nDGCI with NNI indicated best results (R^2=0.92) for the 'Two nodes' growth stage (BBCH scale 32; Meier, 2001) depending on smartphones. The objectives of this study were to investigate the potential of normalized DGCI (nDGCI) in rapeseed (*Brassica napus L.*) for estimating the nitrogen nutrition index (NNI) using visible imagery captured at the canopy scale and to compare these values with those obtained by conventional methods, including NNI and normalized SPAD (nSPAD). Additionally, the influence of two plant factors, namely variety and growth stage, on the correlations among these three variables (NNI, nDGCI and nSPAD) was assessed.

Materials and methods

Field experimental site

The experiments were carried out over two years, during February and April, key vegetation stages for fertilization, and at two different sites: (1) in Toulouse (Occitania, France, GPS: 43°25′53.0″N; 1°43′27.0″E) in 2023 on a clay-limestone deep soil, on a single rapeseed variety (LG Architect) in 20 microplots. Nitrogen was applied in three doses (Figure 1A): the first dose (N1) at the D1 stage (BBCH scale 50), the second dose (N2) at the E-F1 stage (BBCH scale 57-60) and the third dose (N3) was not used. To establish a nitrogen gradient, five N fertilization rates ranging from 0 to 170 kg N.ha-1 (0, 40, 80, 120 and 170 kg N.ha-1) were thus carried out. As no plots were over-fertilized, the plot with the maximum input was taken as the reference.
2) in Savouges (Burgundy, France, GPS: 47°10′15.9″N; 5°03′41.8″E) in 2024 a chalky-clay deep soil, on a mixture of three rapeseed varieties (Dingos, DK exception and Alicia) in 24 microplots. Each total nitrogen dose was divided into three applications (N1, N2 and N3) provided at the C1, D1, and D2 (BBCH scale 53) stages, respectively (Figure 1B). Six nitrogen fertilization modalities ranging from 0 to 250 kg N.ha-1 (0, 50, 100, 150, 200 and 250 kg N.ha-1) were applied. In addition to the experimental micro-plots, over-fertilized subplot was used as control. In the 2024 experiment, the over-fertilized plot received a total nitrogen amount of 250 kg N.ha-1 applied in five doses of 50 kg N.ha-1 each, ten days apart, from February 1 to April 1. For both experiment, to account for local variations between replications, each modality was replicated four times in a randomized design.

Sampling and measurements

In 2023, measurements were carried out on three dates: 8 March, corresponding to the D1 growth stage, and 24 and 26 March, corresponding to the E–F1 stage (Figure 1A). On each observation date, two types of measurements were performed simultaneously on a single observation area (0.13 m×0.175 m) of the microplots: aboveground biomass samples for NNI calculation and RGB photographs taken with a Samsung Galaxy J7 smartphone for DGCI calculation. This observation

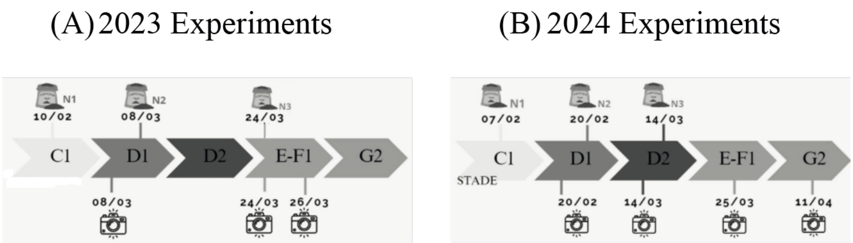

Figure 1. Timeline of nitrogen applications and photographic sessions for (A) the 2023 experiments in Toulouse and (B) the 2024 experiments in Dijon.

area corresponded to the coverage area of a downward-facing camera located 1m above the ground. In order to avoid saturation of the photo sensor at certain points, the photographs were captured under the most uniform lighting conditions achievable. Furthermore, among the shooting parameters, the white balance was set to manual. These included 12 photographs taken on 8 March and 27 photographs taken on 24 and 26 March.
In 2024, the measurements were carried out on four dates (Figure 1B): 20 February (D1), 14 March (D2), 25 March (E–F1) and 11 April (G2). In addition to the previous measurements, SPAD chlorophyll meter measurements were also done. For each observation area, two series of 30 leaf pinch measurements were performed, and an average value was calculated for both series. In order to associate the SPAD measurements with an NNI value, SPAD values were normalized (nSPAD) to the maximal value obtained on an over-fertilized plot (Ravier *et al.*, 2018). RGB photographs were taken with Samsung A23 smartphone with optical parameters similar to those in 2023. For each of the four dates, 24 photographs were taken.

Image processing for nDGCI calculation
From the initial visible image (RGB), a vegetation image was deduced from the procedure described by Gée and Denimal (2020), using appropriate thresholding to extract only the vegetation pixels. The DGCI of each vegetation pixel was calculated from equation found in Rhezali and Lahlali (2017) by changing colour space, from RGB (Red, Green, Blue) to HSB (Hue, Saturation, Brightness). Finally, the DGCI value of the image was calculated as the average of the DGCI values of the vegetation pixels. DGCI values range from 0 (yellow hue, potentially reflecting low N content) to 1 (dark green hue, potentially reflecting high N content).
nDGCI was also calculated as the ratio between the DGCI measurement of the observed plot image and that of an over-fertilized plot. This procedure, which minimizes the impact of lighting variations as well as those internal to the sensor, provides a more reliable indicator. The objective of this novel indicator was to eliminate the need for image calibration using colour charts, facilitating its straightforward application at the canopy scale.
Statistical analyses were performed using R (R Core Team, 2021) and RStudio (2020), an integrated development environment for R.

Results and discussion

A series of vegetation images deduced from image segmentation processing (Gée and Denimal, 2020) are shown in Figure 2, at the four growth stages of rapeseed. Generally, the image processing is quite effective in selecting only vegetation pixels (leaves and stems) while eliminating non-vegetative pixels, such as flowers, soil, residues (e.g., straw and chaff), artifacts like the experimenter's boots, as well as certain pixels that are excessively overexposed to light. Subsequently, for each vegetation pixel, a DGCI value was calculated, and the average of the DGCI values was computed for each vegetation image.
Linear relationships between NNI and nDGCI (for 2023 and 2024 data) or nSPAD (for 2024 data) were then analysed. Subsequently, the impact of two plant factors, which are variety and growth stage, was examined.

Influence of variety and growth stage on linear regression between NNI and nDGCI
Figure 3A shows the 2023 results, which were focused on a single rapeseed variety. It illustrates a linear relationship between NNI and nDGCI, with a coefficient of determination (R^2) of 0.64 across the data from both growth stages. A closer analysis (Figure 3B,C) of these results showed that this value is largely driven by the relationship observed at the E-F1 growth stage (R^2=0.64), which included twice as many data points as the D1 stage (R^2=0.36). For this experiment carried out over one year, it is difficult to conclude about the influence of the growth stage on the quality of the linear regression model due to an insufficient number of measurements.

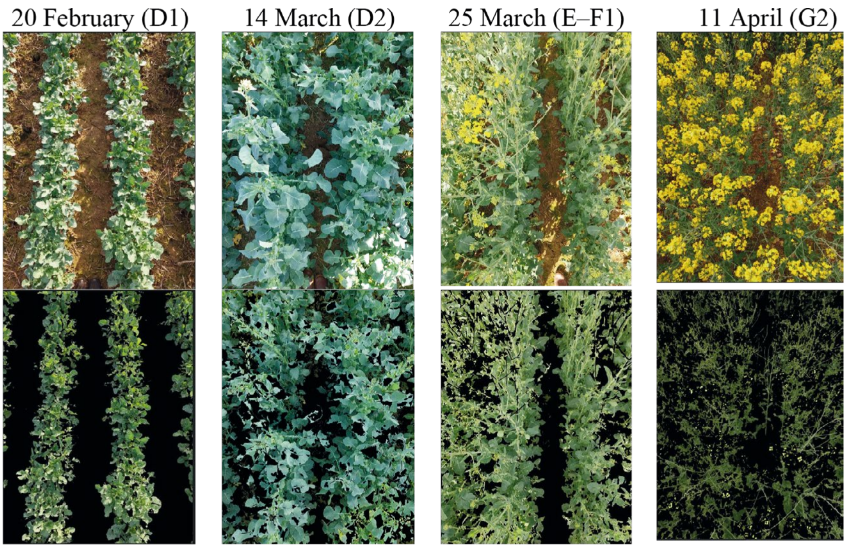

Figure 2. Series of DGCI images derived from image segmentation processing (Gée and Denimal, 2020) from experiments conducted in 2024 at four growth stages of rapeseed (D1 stage, BBCH scale 50; D2 stage, BBCH scale 53; E–F1 stage, BBCH scale 60; G2 stage, BBCH scale 71).

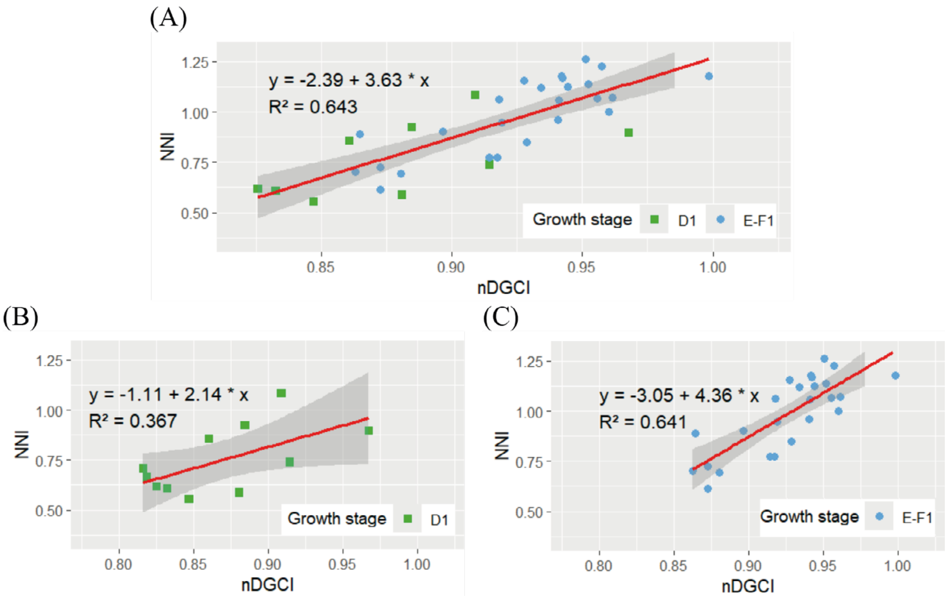

Figure 3. 2023 results with a single rapeseed variety (LG Architect): influence of growth stage on the relationship between NNI and nDGCI.

In 2024, experiments were conducted using a mixture of three rapeseed varieties to reflect common farming practices for pest management. Among these varieties, Alicia blooms earlier than the other two, effectively attracting *Meligethes aeneus* (rape beetle) and helping to protect the later-flowering varieties, namely Dingos and DK exception. The relationship between NNI and nDGCI for this

mixed-variety experiment is presented in Figure 4A, revealing a low-quality linear regression (R^2=0.29) when all growth stages were combined. However, when data were analysed by growth stage, it is noteworthy that the R^2 coefficient values did not improve (ranging from 0.12 and 0.39, data not shown) except at the E–F1 growth stage, where a high-quality linear relationship (R^2=0.63) was observed with the same number of measurements (Figure 4B).
In conclusion, throughout the two-year study, the relationship between NNI and nDGCI demonstrated sensitivity to growth stage. However, at the E–F1 stage, a good correlation was confirmed in both years (R^2=0.64 in 2023 and 0.63 in 2024).

Although the regression equations appeared similar across the two years studied (y=4.36x–3.05 in 2023 and y=4.13x – 3.13 in 2024), analysing the combined dataset from both years (2023 and 2024) deteriorated the quality of the linear relationship (Figure 5), reducing the coefficient of determination (R^2=0.28). This suggests that the relationship between NNI and nDGCI is also influenced by the rapeseed variety, whether grown as a single variety or as a mixture.

Linear model of the relationship between nSPAD and NNI vs nDGCI: influence of the pinched leaf
In the 2024 experiment with a mixture of three rapeseed varieties, the relationships between nSPAD and NNI or nDGCI were also explored. The result of the linear regression observed in Figure 6A demonstrated a low-quality linear regression (R^2=0.29) observed for all data of 2024. When the results were analysed by growth stage, the R^2 coefficient values did not improve (ranging from 0.03

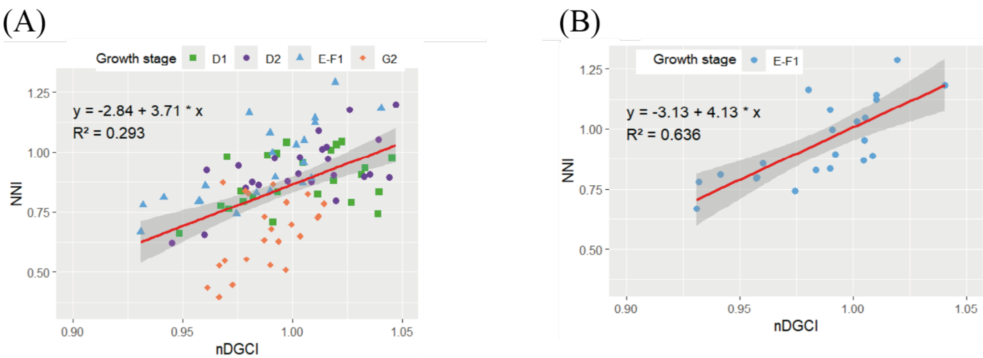

Figure 4. 2024 results. Linear relationship between NNI and nDGCI for a mixture of three rapeseed varieties (Dingos, DK exception and Alicia) (A) at different growth stages (D1, D2, E–F1 and G2), (B) at growth stage E–F1.

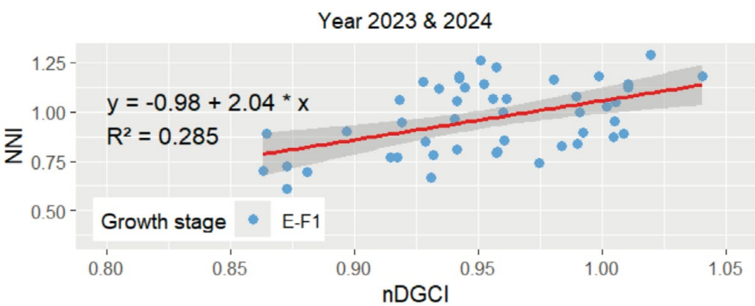

Figure 5. Linear relationship between NNI and nDGCI at the E–F1 growth stage for both years.

to 0.31; data not shown). Moreover, examining the linear relationship between nSPAD and nDGCI, revealed no significant results (p>0.05), with R^2 values ranging from 0.01 to 0.17, depending on the growth stage (Figure 6B). For both NNI and nDGCI variables, no correlation with nSPAD was found. A linear relationship was initially sought because Gée *et al.* (2023) had observed it in a wheat crop using an N-tester chlorophyll meter. However, for this rapeseed experiment, the small range of values obtained for INN and nDGCI did not allow better results to be obtained with other types of relationship (polynomial, exponential). This result raises questions about the measurements obtained with the chlorophyll meter for studying nitrogen content in rapeseed leaves. A number of comments can be made concerning the measurements made during the 2024 campaign. Few articles have evaluated chlorophyll meters for nitrogen fertilization in rapeseed (Dong *et al.*, 2019). In rapeseed, chlorophyll meter measurements are significantly more challenging compared to wheat, as there is no clear reference for selecting the appropriate leaf for measurement, and the leaf is thicker. Based on the research by Dong *et al.* (2019), SPAD measurements at all growth stages were ideally performed on the youngest fully expanded leaf from the top, considering it to represent the highest nitrogen sensitivity. However, precise identification of the leaf and the variety pinched among the three present can be challenging. As a result, measurement uncertainty will therefore be higher, which will impact the reliability of SPAD measurements and, therefore, the relationship between nSPAD values and nDGCI or NNI.

Conclusion

Following two trial campaigns conducted over two years (2023 and 2024) in two different regions, focusing on rapeseed crops with a single variety in 2023 and a mixture of three varieties in 2024, two key findings were observed. First, a linear relationship between NNI and nDGCI was established at the E–F1 growth stage. These results are promising for the use of the nDGCI indicator in rapeseed and partially confirm the potential of visible imagery for estimating NNI in rapeseed crops. Second, the lack of linear relationships between nSPAD measurements with NNI or nDGCI suggests potential issues, requiring a revision of the methodology employed for SPAD measurements. Nevertheless, nDGCI confirms its potential for development in precision agriculture, particularly through the use of drone-based visible imaging.

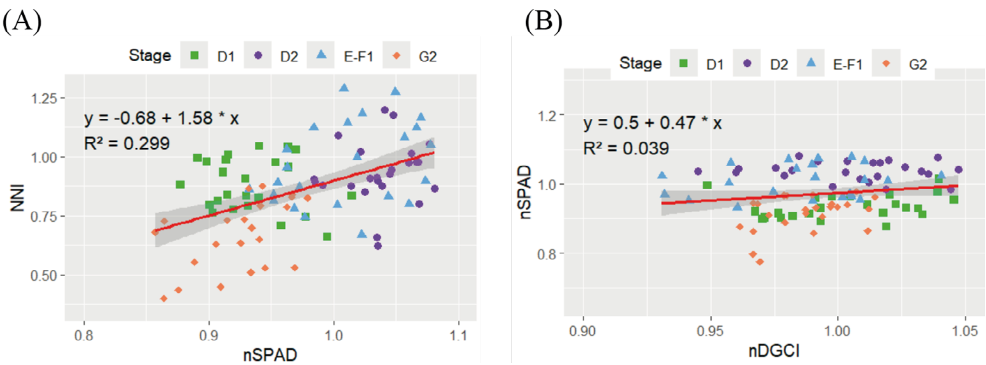

Figure 6. 2024 results. Linear relationship between NNI and nSPAD (A) and nSPAD and nDGCI (B) for a mixture of three rapeseed varieties (Dingos, DK exception and Alicia) at different growth stages (D1, D2, E–F1 and G2).

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