Insights into Entangled Variations in the Red Edge Position and Red to Far-Red Ratios of Soybean Leaves

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Introduction

> Context

• Soybean crops have a strategic importance for food production



• Besides their high protein content, these C3 legumes can fix atmospheric nitrogen for their own growth, which minimizes the use of inorganic fertlizers

- A diverse array of monitoring and management procedures have been proposed to increase the crops' yield while mitigating environmental risks related to:
 - the depletion of fresh water supplies
 - the excessive use of inorganic fertilzers



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• These procedures are often associated with the use of spectral features relating the plants' foliar radiometric parameters to their nutrient (*e.g.*, nitrogen) status

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• This feature is directly associated with the combined effects of light absorption by pigments (*e.g.*, chlorophyll) and scattering by internal foliar structures (*e.g.*, cells)

• The <u>red edge position</u> (*REP*), in turn, corresponds to the wavelength associated with the maximun slope of the red edge, *i.e.*, the peak value of its first derivative



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• The use of this spectral index facilitates comparisons of reflectance data obtained under different measurement conditions and for distinct species

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- The *REP* and the chlorophyll contents of plant leaves are strongly correlated
- Accordingly, the *REP* can be employed, for instance, in the evaluation of crop nutrient status, which can prompt in-season adjustments to fertilizer applications

• Besides nutrient stress, the photosynthetic capacity of soybean leaves may also be affected by other abiotic factors such as distinct light exposure conditions

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• Besides nutrient stress, the photosynthetic capacity of soybean leaves may also be affected by other abiotic factors such as distinct light exposure conditions

 Variations in the red to far red (*R*/*FR*) ratios of light impinging on soybean leaves can trigger shade-avoidance responses (*e.g.*, petiole and internode elongation) to increase light capture

 Since leaves that develop under shade tend to present a reduced photosynthetic capacity, this trait of soybeans also contributes to their photosynthetic efficiency



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• In this work, we assess this possibility through the analysis of the \overline{REP} values and R/FR ratios of soybean leaves with different biochemical characteristics

Materials and Methods



- We used measured reflectance and transmitance datasets provided by the multidisciplinary *LOPEX* (Leaf Optical Properties Experiment, 1996) project
 - Radiometric curves from 400 to 2500 nm, with a resolution of 1 nm
 - Each curve was obtained considering an angle of incidence of 8°
 - Two batches of specimens with markedly distinct chlorophyll (Ch) contents
 - ✤ Batch 1: Typical Cha and Chb contents (2.9 and 0.8 mg/g, respectively)
 - ✤ Batch 2: Low Cha and Chb contents (0.09 and 0.05 mg/g, respectively)

• Reflectance data for representative specimens of Batch 1 and Batch 2

Vis-IR Range

Zoom-in



• Transmittance data for representative specimens of Batch 1 and Batch 2

Vis-IR Range

Zoom-in



Methods

• To obtain the *REP*, we compute the first derivative of the specimens' reflectance curves using a three point numerical differentiation formula:

 $\rho'(\lambda) = (\rho(\lambda+10) - \rho(\lambda-10)) \ge 0.05,$

where $\rho(\lambda)$ denotes the reflectance at a given wavelength (λ)



- To quantify the red to far-red ratios of reflected the light, we use as sampling references the wavelengths that correspond to the chlorophyll absorption peaks
 - under *in vitro* conditions (660 and 730 *nm*): $R/FR_{\rho} = \rho(660)/\rho(730)$
 - under *in vivo* conditions (645 and 735 *nm*): $R/FR_{\rho}^* = \rho(645)/\rho(735)$

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• To quantify the red to far-red ratios of transmitted light, R/FR_{τ} and R/FR_{τ}^* , we replaced the reflectance values by transmittance (τ) values



Batch 1 (typical chlorophyll contents): calculations for reflected light

| Batch 1 | | | | |
|---------------|-------|--------------|------------------|--|
| Leaf Specimen | REP | $R/FR_{ ho}$ | $R/FR_{ ho}^{*}$ | |
| B1a | 710 | 0.1122 | 0.1210 | |
| B1b | 707 | 0.1282 | 0.1399 | |
| B1c | 707 | 0.1285 | 0.1430 | |
| B1d | 709 | 0.1332 | 0.1499 | |
| B1e | 709 | 0.1364 | 0.1479 | |
| Average | 708.4 | 0.1277 | 0.1393 | |

Batch 2 (low chlorophyll contents): calculations for reflected light

| Batch 2 | | | | |
|---------------|-------|--------------|------------------|--|
| Leaf Specimen | REP | $R/FR_{ ho}$ | $R/FR_{ ho}^{*}$ | |
| B2a | 690 | 0.7740 | 0.7759 | |
| B2b | 686 | 0.7860 | 0.7301 | |
| B2c | 685 | 0.7808 | 0.6987 | |
| B2d | 689 | 0.8089 | 0.8062 | |
| B2e | 688 | 0.6619 | 0.5604 | |
| Average | 687.6 | 0.7623 | 0.7142 | |

• Lower *REP* values and higher R/FR_{ρ} and R/FR_{ρ}^{*} ratios

Batch 1 (typical chlorophyll contents): calculations for transmitted light

| Batch 1 | | | | |
|---------------|--------------|------------------|--|--|
| Leaf Specimen | $R/FR_{	au}$ | $R/FR_{	au}^{*}$ | | |
| B1a | 0.0836 | 0.1298 | | |
| B1b | 0.1044 | 0.1544 | | |
| B1c | 0.0782 | 0.1167 | | |
| B1d | 0.0651 | 0.0966 | | |
| B1e | 0.0749 | 0.1140 | | |
| Average | 0.0812 | 0.1223 | | |

Batch 2 (low chlorophyll contents): calculations for transmitted light

| Batch 2 | | | | |
|---------------|--------------|------------------|--|--|
| Leaf Specimen | $R/FR_{	au}$ | $R/FR_{	au}^{*}$ | | |
| B2a | 0.7814 | 0.7741 | | |
| B2b | 0.7882 | 0.7676 | | |
| B2c | 0.7861 | 0.7265 | | |
| B2d | 0.7690 | 0.7640 | | |
| B2e | 0.7450 | 0.6796 | | |
| Average | 0.7739 | 0.7423 | | |

• Higher R/FR_{τ} and R/FR_{τ}^* ratios



Implications for shade-avoidance and chlorophyll monitoring

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• Low *R*/*FR* ratios may act as shade-avoidance signals for soybean leaves

• Consequently, a substantial reduction of their cholorophyll contents followed by an increase in their R/FR ratios can diminish their shade-avoidance responses

• This aspect, in turn, can be detrimental to the plant's photosynthetic capacity and significantly reduce the yield of soybean crops

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- The *REP* is routinely employed as an indicator of a number of factors, notably chlorophyll contents, affecting crop productivity
- Its putative (inverse) connection with the R/FR ratios, upon confirmation, may extend its scope of applications to the monitoring of shade avoidance capabilities



• Conversely, noticeable variations in the R/FR ratios of reflected light could potentially assist the detection of significant reductions in chlorophyll contents



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• We note that the calculation of *R/FR* ratios requires fewer samples (2) than those (3 or 4) usually used in *REP* estimations

Implications for weed management

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 Again, upon confirmation of the REP and R/FR ratios (inverse) connection, REP values could be used as supporting data for weed management strategies





- Implications for intercropping cultivation systems
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- Soybeans are often intercropped with C4 grains (*e.g.*, corn) to increase land-use efficiency and crop yield
- However, intercropped species may affect each other's light exposure conditions in adverse ways
 - *R*/*FR* ratios of light propagated by corn leaves increase as their chlorophyll contents decrease



 Thus, light propagated by chlorophyll-depleted corn leaves may exacerbate the reduction of shade avoidance responses of neighbour soybean plants • We also note that neighbour/partner crops, such as soybean and corn, can have distinct mechanisms of adaptation to stress factors



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- Accordingly, a deeper undertanding about the circumstances in which their REP values can correlate with their R/FR ratios should be pursued
- It would strengthen the foundation required for the design of more cost-effective procedures to evaluate these crops' aggregated and individual health status

Concluding Remarks

> What's next?

• Our findings suggesting an inverse connection between the *REP* values & *R/FR* ratios of soybean leaves with markedly distinct ChI contents need confirmation

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 Given the potential implications of this putative entanglement, future laboratory and field experiments are warranted to evaluate its photobiological basis



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• Our findings suggesting an inverse connection between the *REP* values & *R/FR* ratios of soybean leaves with markedly distinct Chl contents need confirmation

 Given the potential implications of this putative entanglement, future laboratory and field experiments are warranted to evaluate its photobiological basis



• In view of the ever-increasing demand for high-yield and environmentally-friendly crops, such experiments could also be extended to other cultivated plant species

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Thank you!

Questions?