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Chromatic and Numerical Approaches for the Monitoring of Corn Plants under Moderate Water Stress

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ABSTRACT

The increasing scarcity of freshwater supplies, notably due to drought conditions elicited by climate changes, has a detrimental impact in the yield of crops worldwide. Not surprisingly, this type of abiotic stress factor has been the object of extensive studies in plant physiology, precision agriculture and remote sensing fields, just to name a few. Its effects on C_4 species like corn (*Zea mays L.*, maize) tend to follow a cumulative pattern with distinct stages of severity. In its early, moderate, stage, it has a minor impact on the plants' chlorophyll contents. Nonetheless, it is essential to detect and manage it before irreversible damage to the plants' photosynthetic apparatus can occur. The unifacial corn leaves are equipped with stress adaptation mechanisms such as the rearrangement of their chloroplasts. Accordingly, their chromatic attributes can change under moderate water deficit conditions. These changes, however, are often subtle and their assessment can be impaired by varying illumination conditions. Alternatively, multispectral vegetation indices can be employed to assist the monitoring of these plants' water status. In this paper, we address these aspects. More specifically, we investigate the sensitivity of corn leaves' chromatic attributes to changes in their optical properties in response to moderate water stress. Furthermore, we propose an alternative index to assist the monitoring of these changes in the visible (photosynthetic) spectral domain. We carried out our investigation using a first-principles *in silico* experimental approach supported by measured data. Our findings are expected to contribute to the advance of the current understanding about moderate water stress related effects on C_4 plants. Such an understanding, in turn, is instrumental for the development of new water-stress monitoring technologies with a higher reliability to cost ratio.

Keywords: corn, adaptation mechanisms, water stress, reflectance, transmittance, color, multispectral index, precision agriculture, remote sensing.

1. INTRODUCTION

The increasing demand for raw materials obtained from corn (*Zea mays L.*, maize), notably aiming at food and biofuel production, can pose serious risks to the environment. In worst case scenarios, a maximization of corn crop yield at any ecological cost can lead to the contamination (*e.g.*, due to the excessive use of fertilizers) and depletion of scarce freshwater supplies, particularly in regions subject to extensive drought periods elicited by climate changes. Viewed in this context, it becomes essential to foster research initiatives that can effectively contribute to the enhancement of corn cultivation practices without incurring in negative consequences to the surrounding ecosystems. In recent years, a considerable number of these initiatives have been directed toward the cost-effective monitoring of variations in the hydration level (water status) of these crops.[1–5]

The assessment of a plant's hydration level has been often associated with the measurement of its leaves' relative water content. According to Loreto *et al.*,[6] when this quantity falls below 70% of its normal level, it is accompanied by an accentuated reduction in the foliar amount of photosynthetic pigments, notably chlorophylls *a* and *b*. This, in turn, decreases the absorption of light in the visible (photosynthetic) spectral domain, resulting in higher foliar reflectance. Eventually, this process may lead to an irreversible damage of the foliar photosynthetic apparatus and the plant's death. Hence, to avoid those irreversible effects, it is essential to effectively detect and monitor the plant's water stress while it is still at a moderate level (foliar relative water content not lower than 70% of its normal level).

Monocotyledonous C_4 plant species characterized by unifacial leaves,[7] such as corn, are known to present sophisticated mechanisms for adaptation to adverse environmental factors, including water stress.[8,9] In fact,

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measurements performed by Maracci *et al.*[10] on corn leaves whose water content was reduced by ≈25% using an *in vivo* procedure (by withholding water from the soil), showed a decrease in their reflectance in the visible (photosynthetic) spectral domain even though their pigment contents remained relatively unaltered. This was consistent with earlier observations by Thomas *et al.*[11] indicating that the leaves of plants under moderate water stress may appear darker than leaves of plants not stressed because the former reflect and transmit light differently. Thomas *et al.*[11] also mentioned that water stress under *in vivo* conditions (without detaching the leaves from the plant) may decrease foliar reflectance.

It has been proposed[12, 13] that water deficit signals may trigger the migration of chloroplasts away from the corn leaves' mesophyll cell walls. This, in turn, may result in a more homogeneous distribution of these chlorophyll containing organelles. Such a distribution pattern would increase the probability of light detour effects,[14–16] and consequently increase light absorption in the visible spectral domain.[15, 17–19] This cause-effect phenomenon could explain the reflectance decrease observed during moderate water stress under *in vivo* conditions.

A number of non-destructive and relatively low cost procedures can be used to detect and monitor moderate water stress under *in vivo* conditions. Loosely speaking, they can be classified as chromatic or numerical. The former are based in the visual inspection of changes in the chromatic attributes (*e.g.*, hue, lightness ...) related to their perceived colors. The latter are based on the computation of indices using the spectral signatures (reflectance, transmittance and/or absorptance) of those leaves. An index can be defined as a number, or a ratio, calculated from a series of observations (or measurements) and used as an indicator of a certain biophysical process such as water stress. In the case of plant leaves, these numbers, which are normally referred to as multispectral vegetation indices,[2] are usually calculated using radiometric quantities, notably reflectance, measured at specific wavelengths.

In this paper, we investigate the effectiveness of these procedures using an *in silico* (computational) approach supported by measured data. This approach allowed us to perform controlled observations of the effects of moderate water stress on selected specimens of corn leaves. We then used those observations to examine to what extent the water stress effects can be identified using the leaves' chromatic attributes and multispectral indices. For this second component of our investigation, we employed a novel multispectral index, termed MWSI (moderate water stress index), which is proposed in this work, along with two existing multispectral indices that can be used in the monitoring of plant's water status, namely WRDI1 (water deficiency reflectance index 1) and PRI (photochemical reflectance index).[2] While the former has been specifically formulated to assess water stress conditions,[20] the latter has an indirect connection to these conditions through their effects on the plants' photosynthesis efficiency.[21]

2. INVESTIGATION FRAMEWORK

To carry out our *in silico* experiments, we employed the first-principles hyperspectral model of light interactions with unifacial plant leaves, known as ABM-U,[22] and actual specimen characterization data provided in the literature. These experiments involved the computation of directional-hemispherical reflectance and transmittance curves for corn leaves in fresh (turgid) and wilted (moderate water stress) states, the analysis of leaf's chromatic attributes associated with these hydration states and the calculation of spectral indices aimed at their monitoring.

Within the ABM-U's' ray-optics algorithmic formulation, a ray interacting with the tissues of a leaf specimen can be linked to any wavelength (λ) within the spectral domain of interest, which in this investigation corresponds to the 400-700 *nm* region of the light spectrum. For consistency, we adopted a spectral resolution of 5 *nm* in all modeled radiometric curves presented in this work, which were obtained using a virtual spectrophotometer.[23] In their computation, we considered an angle of incidence of 8° and employed 10^6 sample rays (per λ).

Readers interested in the ABM-U's predictive capabilities are referred to related publications.[12, 22] In those papers, the modeled outcomes were quantitatively and qualitatively evaluated through comparisons with measured data and actual observations of photobiological phenomena involving unifacial plant leaves. To enable the confirmation of those evaluations and the reproduction of the findings reported in this work, we have made ABM-U available for online use.[24, 25]

Parameter	A1	A2	B1	B2
Thickness (cm)	0.02040	0.01860	0.02240	01560
Mesophyll percentage (%)	80	80	80	80
Chlorophyll A concentration (g/cm^3)	0.00290	0.00328	0.00342	0.00367
Chlorophyll B concentration (g/cm^3)	0.00080	0.00090	0.00120	0.00129
Carotenoids concentration (g/cm^3)	0.00066	0.00075	0.00091	0.00098
Protein concentration (g/cm^3)	0.05793	0.07407	0.06656	0.07627
Cellulose concentration (g/cm^3)	0.05804	0.07421	0.07152	0.08197
Lignin concentration (g/cm^3)	0.00661	0.00845	0.00760	0.00870
Cuticle undulations aspect ratio	10	10	10	10
Epidermal cell caps aspect ratio	5	5	5	5
Spongy (mesophyll) cell caps aspect ratio	5	5	5	5

Table 1: Summary of ABM-U parameters employed in the characterization of the selected corn specimens in their fresh (turgid) state.

Parameter	A1	A2	B1	B2
Thickness (cm)	0.01632	0.0148	0.01792	0.01248
Mesophyll percentage (%)	80	80	80	80
Chlorophyll A concentration (g/cm^3)	0.00277	0.00313	0.00323	0.00352
Chlorophyll B concentration (g/cm^3)	0.00076	0.00086	0.00115	0.00124
Carotenoids concentration (g/cm^3)	0.00063	0.00071	0.00087	0.00093
Protein concentration (g/cm^3)	0.07389	0.09425	0.08490	0.09728
Cellulose concentration (g/cm^3)	0.07403	0.09443	0.09124	0.10454
Lignin concentration (g/cm^3)	0.00844	0.01076	0.00969	0.01110
Cuticle undulations aspect ratio	10	10	10	10
Epidermal cell caps aspect ratio	5	5	5	5
Spongy (mesophyll) cell caps aspect ratio	6	6	6	6

Table 2: Summary of ABM-U parameters employed in the characterization of the selected corn specimens in their wilted (moderate water stress) state.

In our investigation, we considered four fresh corn leaf specimens, henceforth referred to as A1, A2, B1 and B2, obtained from distinct corn plants (A and B). These specimens were employed in reflectance and transmittance measurements whose results were made available in the LOPEX database[26] along with the corresponding foliar data (*e.g.*, pigment contents, fresh weight and thickness). The ABM-U parameter values used to characterize the four selected specimens in their fresh state are provided in Table 1. For conciseness, the complete description of the procedures employed to obtain the specimens' characterization data, including their bibliographic sources, is provided elsewhere.[12, 13]

The spectral curves computed for the specimens in their wilted state were obtained considering a 25% water reduction in their tissues. This reduction was accompanied by minor changes in their pigment contents as reported in the experiments by Maracci *et al.*[10] involving corn leaves under *in vivo* moderate water stress. Furthermore, measurements performed by Wolley[27] on corn leaves indicate that such a water content reduction is followed by approximately a 20% reduction in thickness and a 2% reduction in area. We accounted for these variations as well as a 25% reduction on the specimens' fresh weights. We also increased the aspect ratio of their mesophyll (spongy) cell caps to account for their resulting flattening[22, 28]. The ABM-U parameter values used to characterize the four selected specimens in their wilted (moderate water stress) state are provided in Table 2. Again, for conciseness, the complete description of the derivation of these parameters values, including supporting bibliographic sources, is provided elsewhere.[12, 13]

We remark that the corn leaves' chloroplasts usually remain arrayed along the mesophyll cell walls.[14,29] Accordingly, light can be propagated without encountering these organelles, a phenomenon known as the sieve effect[14–16,29] that decreases the probability of light absorption (in comparison with a homogeneous chlorophyll solution). On the other hand, during moderate water stress under *in vivo* conditions, the mesophyll chloroplasts may adopt a more homogeneous intracellular distribution triggered by water deficit signals,[12,13] resulting in an intensification of light detour effects[14–16] that increase the probability of light absorption.[15,17–19]

To account for these phenomena, ABM-U adjusts the propagation (scattering) angle of the rays traversing the leaf's mesophyll tissue. This adjustment is performed using a bound derived from applied optics experiments[30]. Accordingly, we kept this bound in place (sieve effect set to *on* in the ABM-U online interface[25]) during the simulations involving specimens in their fresh state, and removed it (sieve effect set to *off* in the ABM-U online interface[25]) during the simulations involving the specimens in their wilted state. Additionally, we applied the law of Gladstone and Dale[12] to obtain the spectral refractive index of mesophyll cell walls after the water content reduction. Its impact in the modeled curves, in comparison with use of the default refractive index of the mesophyll cell, was minor, however.

After computing the modeled reflectance and transmittance data as indicate above, we proceeded to the analysis of the different procedures for the monitoring of moderate water stress. We note that, in actual experiment settings, color images or hyperspectral signatures of corn leaves employed in water stress related studies can be acquired either considering only light reflection, *i.e.*, with one of the leaf's (blade) surfaces placed over an opaque material to block light transmission,[2,31] or considering both mechanisms of light propagation (reflection and transmission). We have also accounted for these possibilties. This led to a total of sixteen testing cases: four specimens (A1, A2, B1 and B2), two light propagation behaviours (reflection only and aggregated reflection and transmission) and two hydration states (fresh and wilted).

It is worth noting that, for materials characterized by a predominantly diffuse (Lambertian) behaviour, directional-hemispherical reflectance and hemispherical-directional reflectance factor quantities are equal for a given direction.[32] Considering that the corn leaves' reflectance follows a near-Lambertian behaviour, notably for small angles of light incidence,[33] we took this equivalence into account in our investigation and assumed that directional-hemispherical and hemispherical-directional reflectance quantities can be used interchangeably in the visualization of leaf chromatic attributes. Similarly, considering that unifacial corn leaves are characterized by a structural symmetry[7] and their transmittance follows a near-Lambertian behaviour[33] as well, we also extended this rationale to the selected specimens' transmittance.

Considering the aspects mentioned above, we have computed the chromatic attributes associated with the fresh and wilted specimens' light reflection behaviour using their modeled reflectance curves. Similarly, we have computed chromatic attributes associated with their combined light reflection and transmission behaviour using their aggregated modeled reflectance and transmittance curves. More specifically, the chromatic attributes were computed through the convolution of the spectral power distribution of a standard CIE D65 illuminant (average daylight),[34,35] the modeled spectral data and the broad spectral responses of the human photoreceptors.[35] This last step was performed using a standard CIEXYZ to sRGB color system conversion procedure.[36] The resulting colors were then employed to render leaf swatches through the application of a grayscale texture. Lastly, the swatches were examined to assess the putative visual effects of moderate water stress in the appearance of the selected specimens.

To evaluate the effectiveness of procedures based on the calculation of multispectral vegetation indices and appraise the efficacy of the proposed index, MWSI, we also conducted two series of computations. In the first, we carried out MWSI calculations considering only the specimen's reflectance, and compared the resulting values for the fresh and wilted states with values obtained using the WRDI1 and PRI indices.[2]

We remark that the effects of moderate water stress on corn leaves reflectance tends to be more prominent around 550 nm,[10,12] and have only a minor impact in the blue end of the light spectrum. Taking these aspects into consideration, we defined the proposed MWSI index as:

$$\mathrm{MWSI} = \frac{\rho(550) - \rho(400)}{\rho(550) + \rho(400)}, \tag{1}$$

where $\rho(\lambda)$ denotes the reflectance at the sampled wavelength λ (in nm).

Similarly, the existing WRDI1 and PRI indices are defined as:[2]

$$\text{WRDI1} = \frac{\rho(510) - \rho(560)}{\rho(510) + \rho(560)}, \tag{2}$$

and:

$$\text{PRI} = \frac{\rho(531) - \rho(570)}{\rho(531) + \rho(570)}. \tag{3}$$

In the second series of computations, we repeated the calculation of the spectral indices (Eqs. 1 to 3) using the aggregation of reflectance (ρ) and transmittance (τ) values, *i.e.*, $\rho(\lambda)$ values in Eqs. 1 to 3 were replaced by $\rho(\lambda) + \tau(\lambda)$ values. This enabled us to examine the effectiveness of these indices when both mechanisms of light propagation are accounted for. In the remainder of the paper, we will refer to these indices when computed in this way as MWSI*, WRDI1* and PRI*.

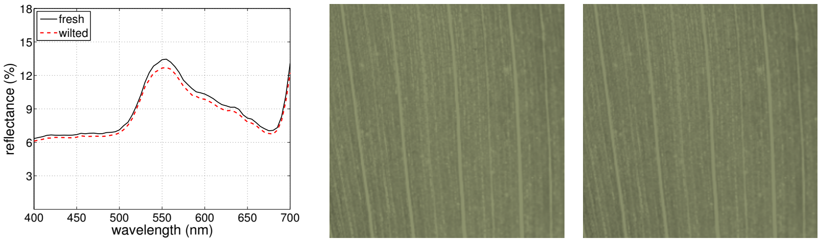
3. RESULTS AND DISCUSSION

In Figs. 1 to 4, we present the outcomes of our *in silico* experiments involving the reflective behaviour of the four selected specimens. As expected,[12,13] a moderate water stress led to a decrease in the specimens' reflectance (Figs. 1a, 2a, 3a and 4a). Moreover, the reflectance decrease was more accentuated around 550 *nm*, which corresponds to the absorption minima of chlorophyll. These observations qualitatively agree with the actual reflectance measurements performed by Maracci *et al.*[10] on water-stressed corn leaves. Besides highlighting the fidelity[37] of the modeled reflectance curves, these aspects provided a sound basis for the generation of the swatches depicting the appearance changes associated with the reflective behaviour of the specimens in their fresh and wilted (moderate water stress) states.

The swatches obtained for the specimens in their wilted state (Figs. 1c, 2c, 3c and 4c) appeared slightly darker than those obtained for their fresh state (Figs. 1b, 2b, 3b and 4b). Similarly, this trend seems to be consistent with empirical observations reported by Thomas *et al.*[11] on the darker colors of water-stressed leaves. However, it is worth noting that the color differences observed in our test cases are quite subtle. Although one may argue that such differences can be more noticeable for different leaf specimens, our observations suggest that a visual inspection of leaf chromatic attributes may not be a reliable indicator of moderate water stress when only the light reflected by the target specimens is considered.

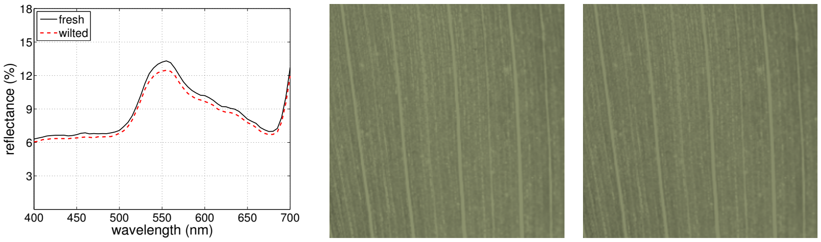
In Figs. 5 to 8, we present the outcomes of our *in silico* experiments involving the aggregated reflective and transmissive behaviour of the four selected specimens. As it can be observed in graphs presented in Figs. 5a, 6a, 7a and 8a, more light is propagated (reflected and transmitted) by the specimens in their wilted state. Moreover, the increase in light propagation is more noticeable in the green region (500 to 600 *nm*) of the light spectrum, and less prominent in the blue region (400 to 500 *nm*). Since the specimens in their wilted state presented a lower reflectance than that obtained for their fresh state (Figs. 1a, 2a, 3a and 4a)), the observed increase in reflected and transmitted light can be directly linked to the increase in specimens' transmittance.

To the best of our knowledge, actual transmittance measurements for corn specimens under *in vivo* moderate water stress have not been reported in the literature to date. Nonetheless, the opposite moderate water stress effects on reflectance and transmittance may be attributed to a combination of phenomena affecting light propagation within the mesophyll tissue. More specifically, we refer to the combined opposite impacts of thickness reduction and detour effects increase, with the latter being caused by the putative rearrangement (more homogeneous distribution) of chloroplasts.[12] In the case of light reflected within the mesophyll, the increase in detour effects on light traversing it back and forth could compensate for the thickness reduction. However, in the case of light transmitted through the mesophyll, the impact of these effects would not be sufficient to compensate for the lower probability of light absorption resulting from the mesophyll thickness reduction.



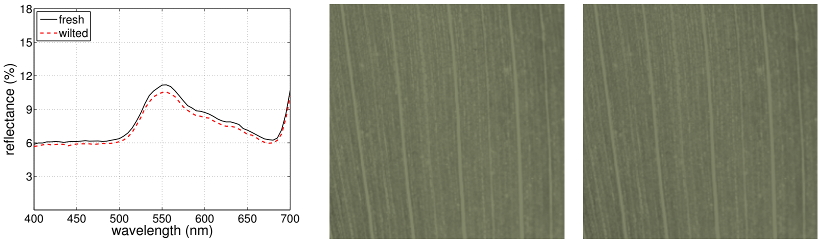
(a) (b) (c)

Figure 1: Light reflected by leaf specimen A1 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



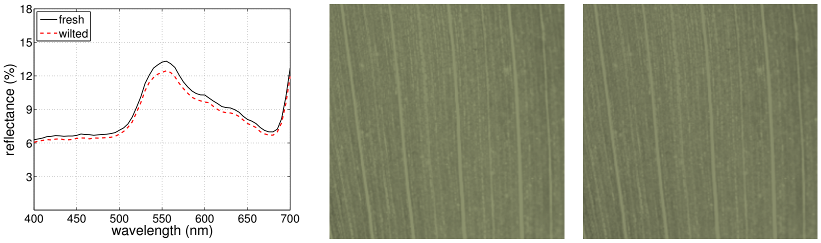
(a) (b) (c)

Figure 2: Light reflected by specimen A2 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



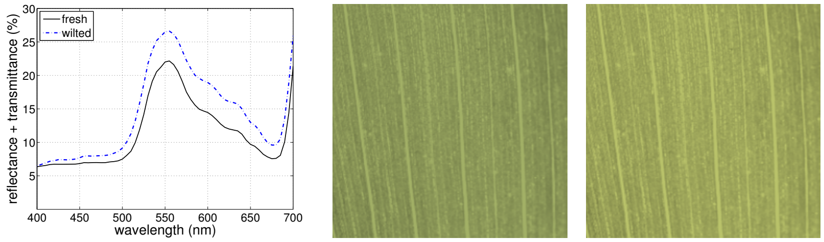
(a) (b) (c)

Figure 3: Light reflected by specimen B1 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



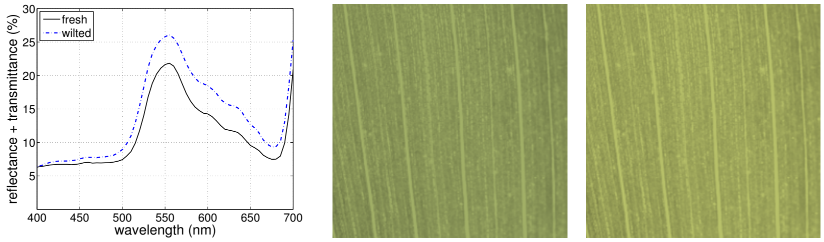
(a) (b) (c)

Figure 4: Light reflected by specimen B2 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



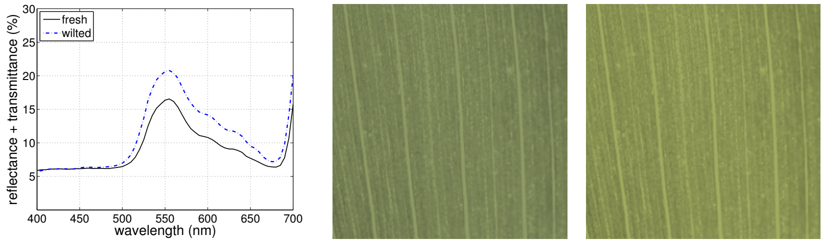
(a) (b) (c)

Figure 5: Light reflected and transmitted by specimen A1 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



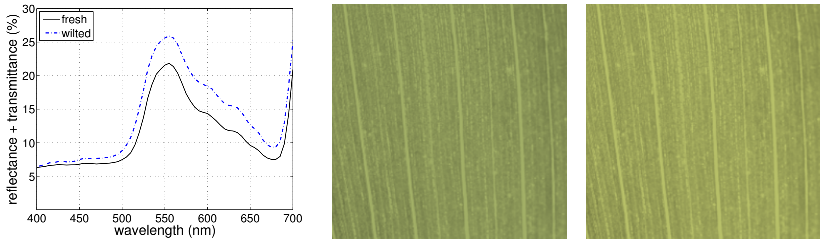
(a) (b) (c)

Figure 6: Light reflected and transmitted by specimen A2 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



(a) (b) (c)

Figure 7: Light reflected and transmitted by specimen B1 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.



(a) (b) (c)

Figure 8: Light reflected and transmitted by specimen B2 in the fresh and wilted (moderate water stress) states. (a) Spectral curves. (b) Fresh state swatch. (c) Wilted state swatch.

	WRDI1			PRI			MWSI		
Specimens	fresh	wilted	δ_{f-w}	fresh	wilted	δ_{f-w}	fresh	wilted	δ_{f-w}
A1	0.2570	0.2495	0.0075	0.0297	0.0226	0.0071	0.3578	0.3487	0.0091
A2	0.2555	0.2494	0.0061	0.0236	0.0208	0.0028	0.3537	0.3439	0.0098
B1	0.2317	0.2212	0.0105	0.0283	0.0249	0.0034	0.3102	0.2982	0.0120
B2	0.2593	0.2540	0.0053	0.0259	0.0235	0.0024	0.3559	0.3411	0.0148

Table 3: Values computed for the spectral indices WRDI1, PRI and MWSI considering the specimens in the fresh and wilted (moderate water stress) states. The differences (δ_{f-w}) between the values obtained for the fresh and those obtained for the wilted state are also included.

	WRDI1*			PRI*			MWSI*		
Specimens	fresh	wilted	δ_{f-w}	fresh	wilted	δ_{f-w}	fresh	wilted	δ_{f-w}
A1	0.4272	0.3976	0.0296	0.0476	0.0336	0.0140	0.5517	0.6069	-0.0552
A2	0.4257	0.4001	0.0256	0.0607	0.0482	0.0125	0.5478	0.6023	-0.0545
B1	0.3877	0.4248	-0.0371	0.0502	0.0456	0.0046	0.4714	0.5636	-0.0922
B2	0.4305	0.4074	0.0231	0.0507	0.0400	0.0147	0.5470	0.6005	-0.0535

Table 4: Values computed for the spectral indices WRDI1*, PRI* and MWSI* considering the specimens in the fresh and wilted (moderate water stress) states. The differences (δ_{f-w}) between the values obtained for the fresh and those obtained for the wilted state are also included.

As a direct consequence of the substantial increase in light propagation brought about by moderate water stress under *in vivo* conditions, the swatches obtained for the specimens in their wilted state (Figs. 5c, 6c, 7c and 8c) appeared markedly brighter than those obtained for the specimens in their fresh state (Figs. 5b, 6b, 7b and 8b). Although these observations refer to specific viewing and illumination geometries, they indicate that the transmission of light through the leaves can have a significant impact on their chromatic attributes. This aspect should not be overlooked when monitoring the water status of corn leaves using procedures based on the visual inspection of their chromatic attributes, either *in situ* or through photographs.

Subsequently, we computed the values for the WRDI1, PRI and MWSI multispectral indices using the modeled reflectance readings obtained for the specimens in their fresh and wilted states. As it can be verified in the values presented in Table 3, moderate water stress led to a reduction in the magnitude of all three indices. However, while the average difference between the values calculated for the fresh state and the wilted state using WRDI and PRI were 0.0074 and 0.0039, respectively, the average difference was 0.0114 using MWSI.

It is relevant to note that well-designed spectrophotometers usually have an uncertainty between ±0.005 and ±0.01 measurement units in the visible spectral domain.[38–40] This aspect should be taken into account in the computation of multispectral indices using measured reflectance values. Accordingly, differences below 0.01 between index values obtained considering distinct leaf hydration levels may hinder the correct interpretation of those values. Thus, the relatively higher differences associated with the computed MWSI values suggest that the proposed index may allow for a stronger degree of confidence in the monitoring of moderate water stress than the other two tested indices when only the target specimens' reflective behaviour is considered.

Finally, we computed the values for the WRDI1*, PRI* and MWSI* indices using the modeled reflectance and transmittance readings obtained for the specimens in their fresh and wilted states. As it can be verified in Table 4, while the PRI* values decreased following a moderate water stress and the MWSI* values increased, the WRDI1* values depicted inconsistent variations. More specifically, while the WRDI* values decreased for specimens A1, A2, and B2, they increased for specimen B1. Such an inconsistency would be detrimental for the reliable monitoring of moderate water stress. With respect to the average difference between the values calculated for the fresh state and the wilted state, it was 0.0114 using PRI*, and significantly higher in absolute terms, 0.0777, using MWSI*. This aspect further illustrates the effectiveness of the proposed multispectral index.

Clearly, these preliminary findings have to be taken into account with a certain degree of caution. A larger number of specimens need to considered in real experimental conditions so that the aforementioned observations can be generalized, particularly with respect to the efficacy of the employed multispectral indices. Nonetheless, the results obtained using only reflectance values (Table 3) indicate that multispectral index differences may be too small in certain cases to allow for a reliable monitoring of moderate water stress under *in vivo* conditions. Although, these differences tend to become more prominent when aggregated reflectance and transmittance values are used (Table 4), they may not always present a consistent variation trend in response to distinct leaf hydration levels.

Despite the preliminary character of the findings reported in this work, we believe that they provide useful insights for future investigations on the monitoring of water stress not only in corn plants, but also in other C_4 species, like surgarcane (*Saccharum officinarum*). These not only share similar characteristics with corn plants (*e.g.*, unifacial leaves) and have a similar importance for the food and biofuel production,[41] but are also associated with serious environmental concerns. Like in the case of corn crops, such concerns also arise from the extensive use of fertilizers and water for irrigation, and their negative impact in ecosystems already under pressure by adverse climate changes.

4. CONCLUDING REMARKS

The reliable monitoring of moderate water stress under *in vivo* conditions is essential for the ecologically sustainable increase of corn crop yield. The preliminary findings reported in this work indicate that the current approaches based on the visual inspection of leaf appearance changes or the computation of multispectral vegetation indices, although promising, still have a relatively limited efficacy.

In silico investigation frameworks can be instrumental for their enhancement by enabling the controlled assessment of a plant's responses to a wide range of environmental stimuli. However, we remark that these frameworks are based on the use of computer models. It has been noted[42] that "all models are wrong", albeit some are useful. Hence, a steeper rate of progress in this area will likely come about once the employed models' usefulness (predictive capabilities) can be fully assessed. To achieve that goal, it will be necessary to obtain more measured data from *in vivo* experiments involving variations in the hydration levels of corn and other similar C_4 plants like sugarcane.

In the last decades, however, relatively little attention has been given to such experiments in comparison with the development of new computer models. The fact that data scarcity makes the verification of the degree of fidelity of these models' predictions difficult is often overlooked, notably with respect to the simulation of phenomena still not completely understood such as the C_4 plants' mechanisms of adaptation to water stress. Viewed in this context, new data acquisition initiatives are not only long overdue, but also fully warranted. Accordingly, such initiatives should receive a stronger level of support from the scientific community.

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