Feasibility of estimating the leaf area index of maize traits with hemispherical images captured from unmanned aerial vehicles

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Abstract. *Feeding a global population of 9.1 billion in 2050 will require food production to be increased by approximately 60%. In this context, plant breeders are demanding more effective and efficient field-based phenotyping methods to accelerate the development of more productive cultivars under contrasting environmental constraints. The leaf area index (LAI) is a dimensionless biophysical parameter of great interest to maize breeders since it is directly related to crop productivity. The LAI is defined as the one-sided photosynthetically active leaf area per unit ground area. Direct estimates of the LAI through leaf collection and subsequent leaf area determination in the laboratory are tedious and time-consuming. Hence, indirect methods based on gap fraction theory are frequently used for in situ LAI estimation. The LAI obtained from gap fraction analysis by most optical sensors available on the market is not the true LAI, but a term called the "effective LAI" that does not consider foliage clumping. Hemispherical images of the bottom-up view of crop canopies offer important advantages to maize breeders, such as a low cost compared to other commercial sensors, and it also may provide LAI estimates corrected for foliage clumping (i.e., true LAI). However, taking bottom-up hemispherical images in every single plot of a maize breeding program can take time and patience. The use of small-sized unmanned aerial vehicles (UAVs) in agriculture has allowed for crop information inference at spatial and temporal resolutions that exceed the benefits of other remote sensing technologies (e.g., airborne, satellites). We assessed the efficacy of using UAVs to collect hemispherical images for estimating the LAI. To do this, we investigated the suitability of using nadir-view hemispherical images taken from a UAV flying at a low altitude (15 m) to accurately derive LAI estimates based on gap fraction analysis in a maize breeding trial carried out near Seville, Spain. Six maize cultivars grown in a split-plot design with three blocks and two irrigation treatments (well-watered and water-stressed) were used in the experiment. LAI estimates from top-down hemispherical imaging taken from the UAV were compared with LAI estimates from both bottom-up hemispherical imaging and direct LAI estimates obtained from an allometric relationship derived in the study. The results show that* *hemispherical images taken from a UAV flying at a low altitude can estimate the LAI of maize breeding plots as accurately as by the classical bottom-up hemispherical imaging approach. CAN-EYE software, which includes automatic image classification and allows the processing of a series of hemispherical photographs, was used in this experiment.*

Keywords. *LAI, precision agriculture, hemispherical images, maize, allometric relationship, UAV*

1. Introduction

In the next few decades, the world's population will continue to grow. To produce sufficient food for all, food production will have to increase at least equally fast (Zipper et. al, 2016). As resources such as land, water and nutrients are limited, this will have to be accomplished by increasing the efficiency of agriculture in a sustainable way (Foley et. al, 2011). This is an unprecedented challenge because the increase has to be attained while dealing with major economic, environmental and societal constraints, such as the scarcity of arable land, droughts in some areas and floods in others, deforestation and carbon dioxide emissions (Foley et al., 2005; Tilman et al., 2011).

Wheat, rice, and maize are the three most valuable cereal crops in the world (Lobell et al., 2013). Maize provides stable food in developing countries and is sometimes the sole source of income for farmers who mainly practice subsistence farming (Shiferaw et al., 2011). However, despite the potential benefits, its cultivation is limited by water supply since it is one of the cereal crops most susceptible to drought (Daryanto et al., 2016; Wossen et al., 2017). When the crop is subjected to water stress, physiological changes occur, and crop yield can be affected (Araus, 2007). A water deficit during grain filling substantially affects yields, although the plant response to drought stress depends on the cultivar and drought level (Grzesiak et al., 2013). Despite the water stress affecting most of the vital functions of the crop, cell turgor, cell expansion rate, cell wall synthesis and leaf rolling are the primary symptoms of drought (Avramova et al., 2015; Min et al., 2016). Leaf rolling reduces the leaf surface, causing less evaporation of water and a lower photosynthetic capacity (Fernandez and Castrillo, 1999; Baret et al., 2017).

Water is the main limiting factor in crop production since drought is the most important abiotic stress that influences plant growth (Basu et al., 2016). Water stress during certain phenologic stages such as flowering and grain filling causes yield losses (Turner, 1990; Metin Sezen et al., 2014). Although tolerance levels and crop responses to drought stress can be different depending on the plant species and the environmental conditions, plant breeding programs are mainly focused on selecting phenotypes with abiotic and biotic stress resistance and, especially, on identifying cultivars with high yields under water stress conditions (Wesley et al, 2002; Maazou et al., 2016; Prasad Vurukonda et al., 2016).

The production of new, improved cultivars with the capacity for significant yield in water stressed environments requires an evaluation under field conditions of the genotypes previously selected in the laboratory (Sareen et al., 2012; Bita and Gerats, 2013). These field trials, in which a large number of cultivars are screened, are time-consuming and use crop yield as the only variable for selecting the best crop varieties (Makanza et al., 2018; Tanksley et al, 1989). Because maize production depends on a large number of factors such as morphological, anatomical and physiological factors, new phenotyping tools and precision methodologies have undergone fast development in recent years (Rahaman et al., 2015). These technological advances will allow breeders to identify factors that influence the behavior and production efficiency of each cultivar (Cobb et al., 2013). This is important to promote the retention of new cultivars with a specific phenotypic response to water stress and, in this way, to accelerate the plant breeding process (Nogué et al., 2016).

Among the phenotypes of interest for monitoring in breeding programs, biophysical crop variables are considered especially important (Danner et al, 2017). These variables are parameters that can provide information about crop health since they are affected by physical and biological factors influenced by the atmosphere, and by soil and plant physical characteristics (Haboudane et al, 2002; Hmida et al., 2017). Biomass, the leaf area index (LAI), plant height, the fraction of vegetation cover (FVC) and the fraction of absorbed photosynthetically active radiation (FAPAR) are some of these variables of interest (Sharifi et al., 2018).

LAI is defined as the leaf area of the canopy per unit area projected on the soil (Watson, 1947; Chen et al., 1992) or as the photosynthetically active leaf area per unit soil (Fuchs, 1984; Bégué, 1993). This index is a key biophysical variable required for crop modeling and is a precise way of estimating the ability of the canopy to intercept incoming photosynthetically active radiation (PAR)

(Delegido et al., 2016; Din et al., 2017). Since carbon is fixed by the interception of radiation and then converted into chemical energy; the LAI can be used as a parameter indicative of the productivity of a crop (Favarin et al., 2002). LAI is affected by abiotic stresses such as drought and is a good tool for evaluating the growth and development of crops in agronomic studies of crop water requirements and evaluations of bio-energetic efficiency (Canfalonieri et al., 2013).

There are many techniques for measuring the LAI (Gower et al., 1999; Küßner and Mosandl, 2000; Hyer and Goetz, 2004; Jonckheere et al., 2004; Weiss et al., 2004;). The two most used LAI measurement methods are direct LAI measurement and indirect LAI estimation (Olivas et al., 2013). Direct methods are destructive because leaf material must be completely collected (Blanco and Folegatti, 2003), one example of this is the Li-3100C (Li-Cor, Lincoln, NE, USA), moreover these methods can be time-consuming and prohibitively expensives when applied to large crop areas (Poblete-Echevarría et al., 2015). Alternatively, in recent years, indirect methods including plant canopy analyzers, hemispherical images, and ceptometers have been developed. These methods utilize the relationship between light transmittance through the canopy and various methods of gap fraction analysis, representing the majority of methods tested for agricultural applications (Peper and McPherson, 2003).

The use of hemispherical photos, also known as fisheye lens images, is based on the estimated position, size, density and distribution of canopy gaps, which characterize the canopy geometry through which the intercepted solar radiation is measured (Jonckheere et al., 2004). This method is more robust than others that measure the gap fraction for different zenithal angles, such as the LAI-2200 and 2200 (Li-Cor, Lincoln, NE, USA), because they may also analyze the gap-size distribution (De Bei et al., 2016).

The development of UAVs (unmanned aerial vehicles), or drone technology, has improved in the last few years, and the benefits of using drones in agriculture are becoming more apparent to farmers and researchers (Candigo et al., 2015). Drone applications in agriculture range from mapping and surveying to crop-dusting and spraying (Dupont et al., 2017). Taking all this into consideration, our working hypothesis is that UAVs may facilitate collecting hemispherical images for LAI estimation. We investigate the suitability of using nadir-view hemispherical imaging taken from a UAV flying at a low altitude to accurately derive LAI estimates based on gap fraction analysis in a maize breeding trial carried out near Sevilla, Spain. The specific objectives, given this approach, were (i) to develop an allometric relationship for non-destructive yet direct estimates of LAI, and (ii) to estimate and compare the LAI from bottom-up and top-down approaches and compare the results.

2. Materials and Methods

2.1. Field description and experimental conditions

The trial was conducted in an experimental maize field (*Zea mays* L.) during the 2017 growing cycle. The field was located 10 km from Seville (lat. 37°27'N, long. 5°58'O). The climate of the study area is Mediterranean with an average rainfall and average annual temperature of 565 mm and 18.5°C, respectively. The soil is loam, with a pH greater than 7, and the top layer of the soil (40 cm) contains 1.3 % total organic matter.

Twenty maize cultivars coded with numbers from 1 to 20 were used. However, only six cultivars (4, 6, 8, 14, 16, and 18) were chosen for this study. Cultivars 4, 6 and 8 were drought tolerant and cultivars 14, 16, and 18 were drought susceptible. The experiment was arranged in a randomized block design with three replications per treatment. Two irrigation treatments, well-watered (WW) and water-stressed (WS), differing in irrigation were evaluated. The plots were irrigated by pressure compensating drippers. The dripper discharge was 2 l h^{-1} and dripper spacing was 0.5 m. The WW treatment received an irrigation allocation of 711 mm and the WS treatment received 353 mm until 13th July when irrigation was cut off, coinciding with grain filling. Soil moisture was measured at six fixed depths (10, 20, 30, 40, 60, and 100 cm) using a PR2 profile probe (PR2/6, Delta-T Devices Ltd., Cambridge, UK). Each experimental plot took up an area of 4.50 m^2 (6.00x0.75), which accommodated 2 maize rows with 35 plants per row (Fig. 1).

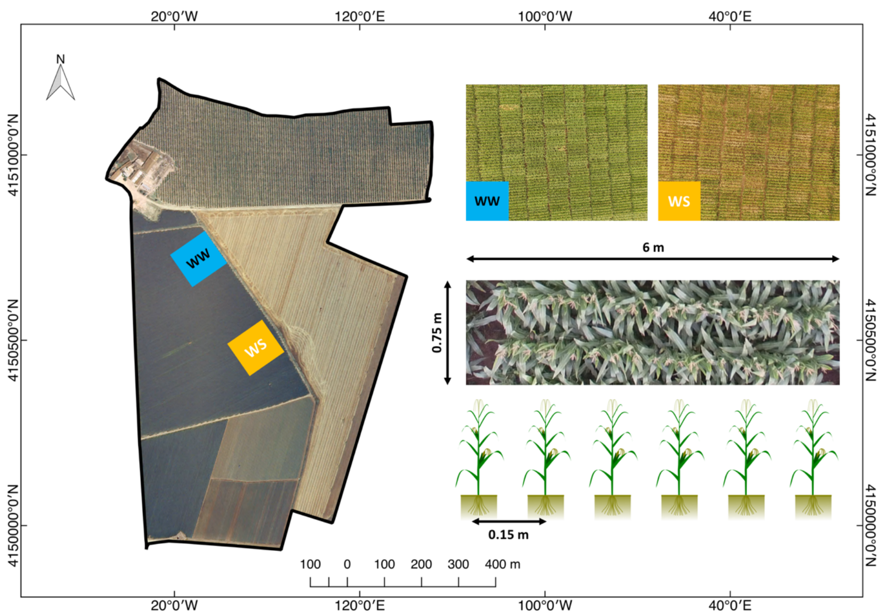

Fig. 1. The experimental setup with details of a single plot and the location of the trial plots.

2.2. Calculation of leaf area index (LAI) by the direct method

To measure leaf area (LA), subsamples of 80 leaves were taken around the perimeter of the trial to avoid affecting plot conditions. Then, the leaves were stored in cooler containers and immediately taken to the laboratory for analyses. For each leaf, the linear dimensions, length (L) and maximum width (W), were measured. The areas of the leaves were measured with a leaf area meter (LI-COR 3100; Lincoln Inc., Nebraska). An allometric relationship to derive the leaf area of individual leaves from L and W measurements was developed.

During the month of July at the same time that top-down and bottom-up hemispherical images were taken in all plots, five plots from the WW treatment were selected. Two representative plants from each plot were selected for measurement of the width and length of all their leaves with a flexible tape graduated in mm, these dimensions were used to calculate the leaf area of each leaf using the abovementioned allometric relationship. The total leaf area for each plant was calculated as the sum of unitary leaf areas for all plant leaves. The LAI for the five plots was obtained according to the following expression:

LAI = $\frac{PLA\ (m^2)}{PD\ (m^2)}$, where PLA is the plant leaf area and PD is the plant density.

2.3. Photography Acquisition

Hemispherical bottom-up images were taken in JPEG format at the highest possible resolution (4000x3000 pixels) with a GoPro Hero 3 black edition (Go Pro Inc., San Mateo, California, USA) equipped with a fisheye lens effect at ground level from the center of each plot. Up-bottom images were captured with a DJI Phantom 3 with an integrated 14-megapixel camera mounted on a motorized stabilization gimbal that allows changes to the viewing angle and dampens rotor-induced vibrations. Images were captured at 15 m above ground level with a resolution of 4000x3000 pixels. Cameras were not calibrated using the method recommended in https://www6.paca.inra.fr/can-eye/ since each was considered to have a perfect fisheye lens where the optical center of the ''camera–fisheye lens'' corresponds with the center of the image.

2.4. Image processing

2.4.1. Digital hemispherical photography (DHP) for LAI estimates

Methods based on DHPs are becoming increasingly popular as an alternative solution to all current noncontact instruments for FVC and LAI measurements (Chen et al. 2006). Hemispherical images capture the light obstruction/penetration patterns in the canopy, from which the canopy architecture and foliage can be quantified (Demarez et al., 2008; Weiss et al., 2004). These methods transform each pixel position from images into angular coordinates and are then able to distinguish leaves from the background (i.e., sky or soil) to calculate gap fraction (Zhang et al., 2005). The gap fraction of a canopy is the fraction of view that is unobstructed by the canopy in any particular direction and it is described by the Poisson distribution (Welles et al., 1996; Gardingen et al., 1999). This method assumes a random spatial distribution of leaves in the canopy. However, not all canopies have random distribution of leaves, producing variations between the true LAI and the LAI, known as effective LAI, obtained using inversion methods of the type employed in this work. An advantage of DHP, against other indirect methods, is that the overlapping effect of leaves, the so-called clumping effect (Ryu et al., 2010), may be estimated and true LAI approached.2.4.2. CAN-EYE software

Leaf area estimates from images were processed using the CAN-EYE software. This software is an imaging software, available just for Windows, used to extract the canopy structure characteristics from RGB images (either acquired with a fish-eye or with a classic objective). CAN-EYE software is able to compute the effective LAI and several estimates of the true LAI by adjusting a clumping index (Weiss et al., 2004) based on the Lang and Xiang (1986) averaging method.

Hemispherical bottom-up images were analyzed directly using the software. However, top-down images were segmented with Adobe Photoshop software to select each plot from images taken from the UAV. One of the advantages of CAN-EYE software is that it uses a tool to mask areas to eliminate parts of the images contaminated by undesirable objects (dried leaves, sun glint, etc.), which are often present when acquiring downward-facing images. The CAN-EYE V6.4.7 release was used for this study.

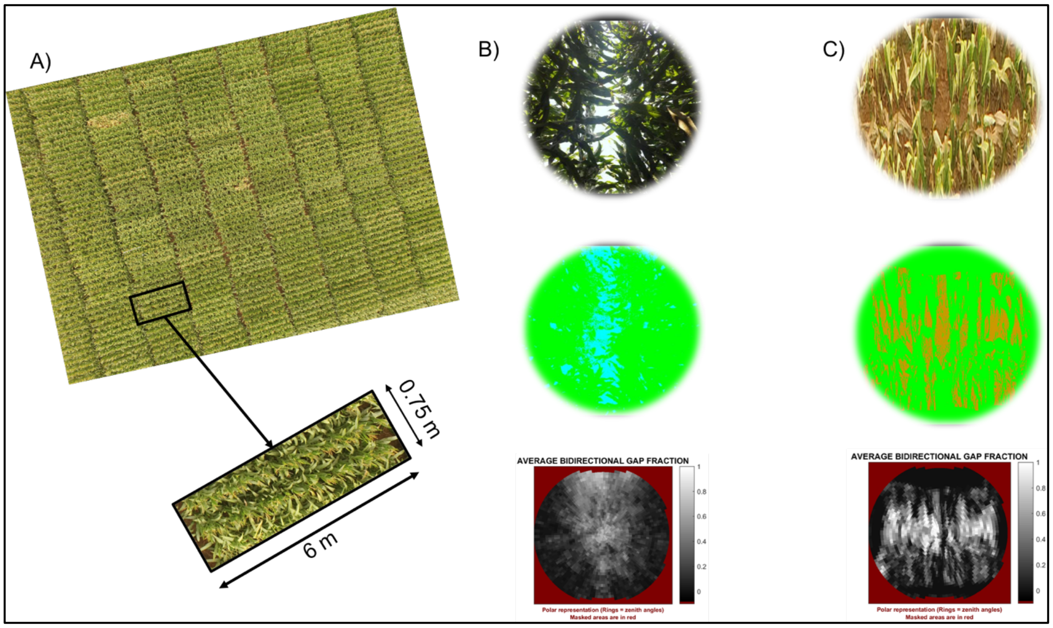

Fig. 2. Description of image processing. (A) Images segmentation process with Adobe Photoshop. (B) CAN-EYE classifications of bottom-up images and (C) CAN-EYE classifications of top-down images

2.5. Statistical analyses

LAI values estimated with direct and indirect methods were compared with the following error measures: the root mean square error (RMSE), the mean absolute error (MAE) and the Nash-Sutcliffe efficiency (NSE), which were calculated using the following expressions:

$$MAE = \frac{1}{N}\sum_{i=1}^{N}|O_i - S_i| \quad RMSE = \sqrt{\frac{\sum_{i=1}^{N}(O_i - S_i)^2}{N}} \quad NSE = 1 - \frac{\sum_{i=1}^{N}(O_i - S_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2}$$

The RMSE and MAE represent the average differences between the model predicted values (S) and the observed (O) values. However, it is a normalized statistic that determines the relative magnitude of the residual variance ("noise") compared to the measured data variance ("data or information"). It is important to include these absolute error measures in model evaluation because they provide an estimate of model error in the units of the variable. The MAE provides a more robust measure of average model error than the RMSE since it is not influenced by extreme outliers (Legates and McCabe, 1999).

3. Results and discussion

3.1. Soil water content

The temporal variations of soil water content (θ_v) for the WS and WW treatments are shown in Fig. 3. The values shown correspond to the average θ_v for the 0-60 cm soil profile, the active root-zone for this crop species. Moisture values for the WW treatment were relatively stable (0.35-0.40 $m^3\ m^{-3}$) for all cultivars and slightly above field capacity ($\approx$0.3 $m^3\ m^{-3}$) as determined by the Saxton et al. (1986) pedotransfer functions. The θ_v values of the WS treatment decreased during the water stress period, especially for cultivar 4.

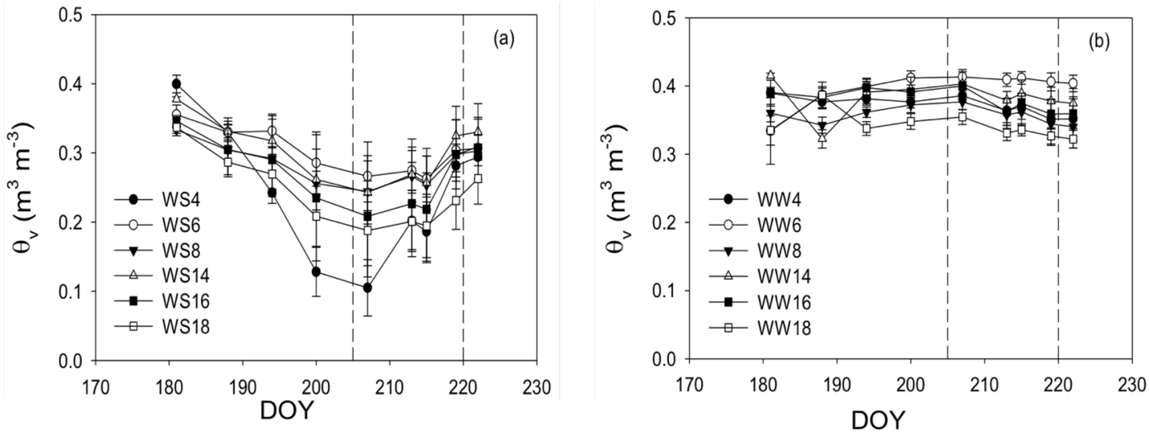

Fig. 3. Temporal variation in soil water content (θ_v) for the six cultivars tested in the WS (a) and WW (b) treatments. Values shown correspond to average depths between 0 and 60 cm. The vertical bars represent the mean standard error (n=3). Discontinuous lines show the period when hemispherical images were taken. The label for each cultivar (4, 6, 8, 14, 16, and 18) is preceded with WW or WS, indicating the irrigation treatment.

3.2. Allometric relationships

Obtaining an allometric relationship that allows estimating the leaf area of individual maize leaves in a non-destructive way, is of great interest for the validation of other non-destructive methods in trials where the plant material cannot be sampled, as it is the case of a trial of varieties where the variation in the number of plants per sub-plot would alter the productivity results of the varieties. In this paper, an empiric relationship with the capacity to explain 96% of the variability in observed single leaf area was obtained (Fig. 4). The relationship is a first-degree equation, y = ax, where the independent variable is calculated as the product of W and L, being 0.7678 the slope of the linear relationship. A similar relationship was reported by Birch et al. (2003), although the value they obtained was 0.75, slightly lower than the value found in this study.

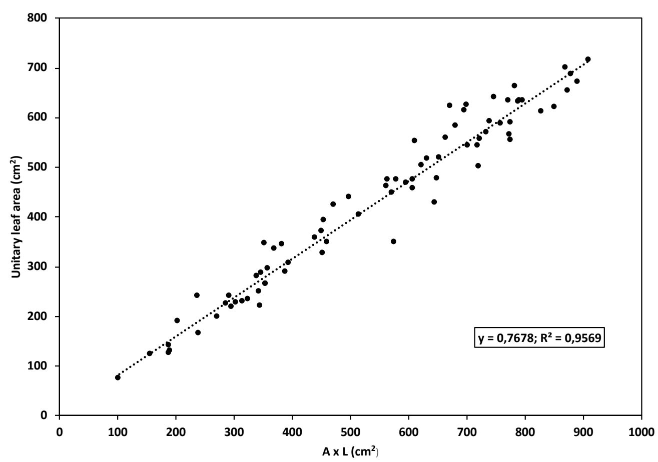

Fig. 4. The allometric relationship obtained from leaf dimensions of a plant and unitary leaf area: W: maximum leaf width; L: maximum leaf length.

3.3. Validation of LAI estimates using hemispherical images

The reliability to estimating the LAI of maize from hemispherical images through gap fraction analyses using CAN-EYE software was assessed in five WW treatments. LAI estimates with the direct method (LAI-d) using an allometric relationship and LAI with the indirect method (LAI-i) are shown in Fig. 5. LAI-i and LAI-d values were similar, with mean values ranging from 6.1 to 5.9 m^2 m^2. respectively. A 3-4% difference was observed between LAI-i and LAI-d. This small difference may be explained by the fact that, in the direct method, only leaves were measured, while in the indirect method other plant organs (e.g. stems) are also considered. For that reason, LAI-i values are known by some researchers as the plant area index (PAI) (Holst et al., 2004; Sandmann et al., 2014). In any case, both LAI-d and LAI-i values are similar to those observed by Song et al.

(2010) in well-watered maize plants.

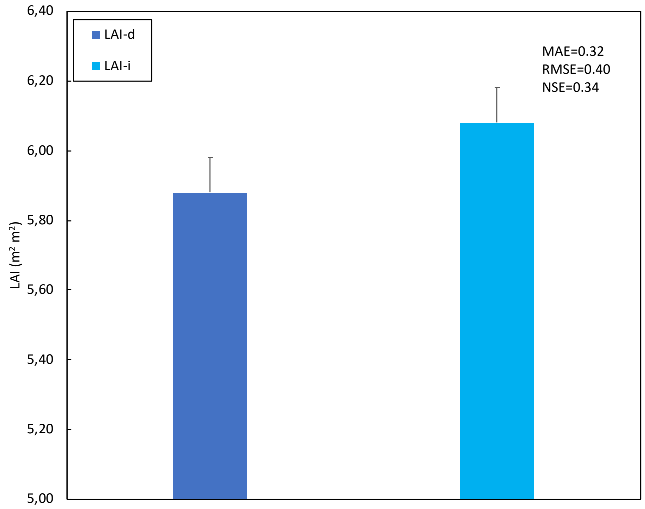

Fig. 5. Comparison of LAI values estimated using the direct method (LAI-d) and indirect method (LAI-i). The vertical bars represent the mean standard error (n=5). MAE, RMSE and NSE are statistics used to analyze the estimation error when the direct method is used to estimate LAI. MAE: mean absolute error; RMSE: root mean square error; NSE: Nash–Sutcliffe model efficiency coefficient.

3.4. Estimating LAI using a UAV

LAI values for six cultivars (during DOY: 213) were compared using images taken from a UAV (top-down and at ground level (Fig. 6). LAI values were similar for both methods.

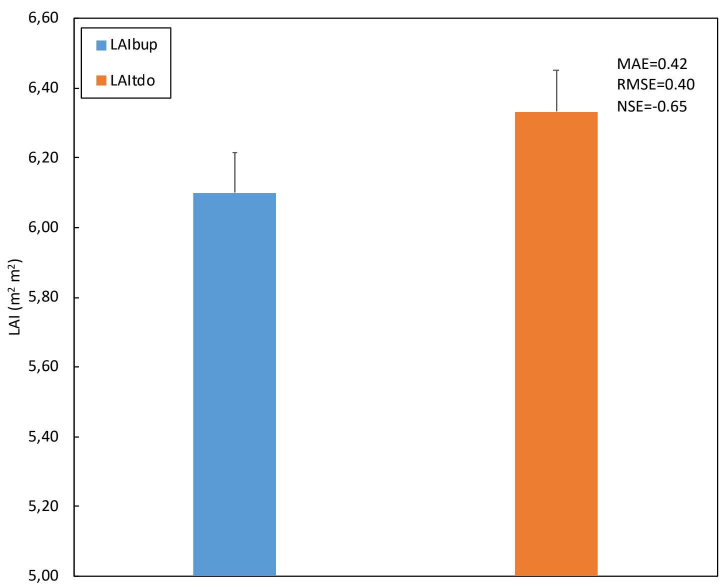

Fig. 6. Comparison of LAI values using top-down (LAItdo) and bottom-up (LAIbup) images. The vertical bars represent the mean standard error (n=6). MAE, RMSE and NSE are statistics used to analyze the estimation error when the direct method is used. MAE: mean absolute error; RMSE: root mean square error; NSE: Nash–Sutcliffe model efficiency coefficient.

When mean LAI values for each cultivar were statistically compared using Duncan's multiple range test (Fig. 7), significant differences were not found between the LAI estimated using up-bottom images (LAIbup) and the LAI estimated using top-down images (LAItdo). This shows that it is possible to estimate LAI using gap fractions obtained from hemispherical images from UAVs.

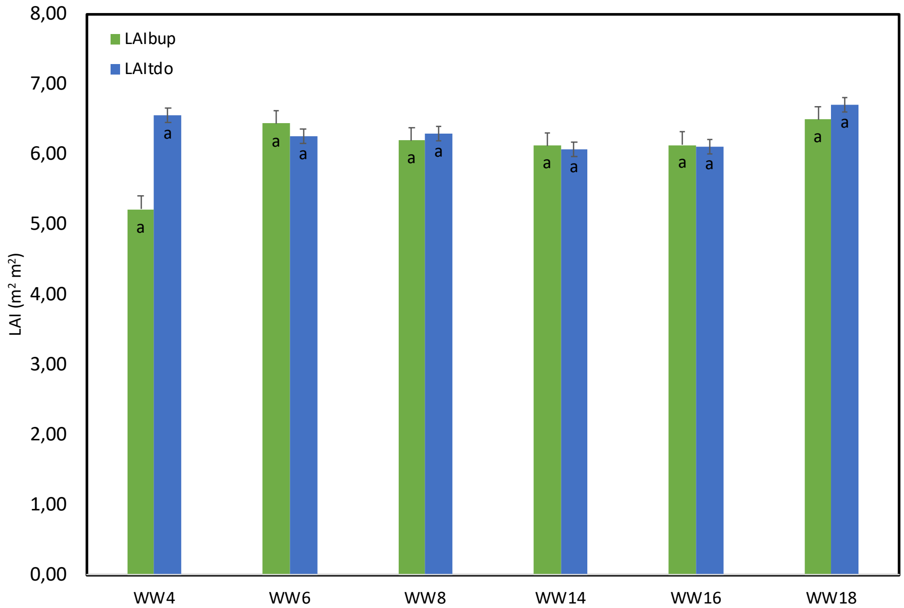

Fig. 7. LAI values using bottom-up (LAIbup) and top-down (LAItdo) images for six cultivars. The vertical bars represent the mean standard error (n=6). Different letters indicate statistically significant differences as measured by the Duncan test (P < 0.05).

3.5. Biophysical variable responses (LAI and VCF) to water stress during grain filling

The temporal variation of biophysical variables (LAI and VCF) for the WW and WS treatments are shown in Fig. 8. Values have been obtained as the mean of six cultivars. Due to water stress, the WS LAI and VCF decreased from 20% and to 40%, respectively, in contrast to the WW treatment. In previous studies, it was demonstrated that the LAI and VCF are closely related, although nonlinearly (Nielsen et al., 2012).

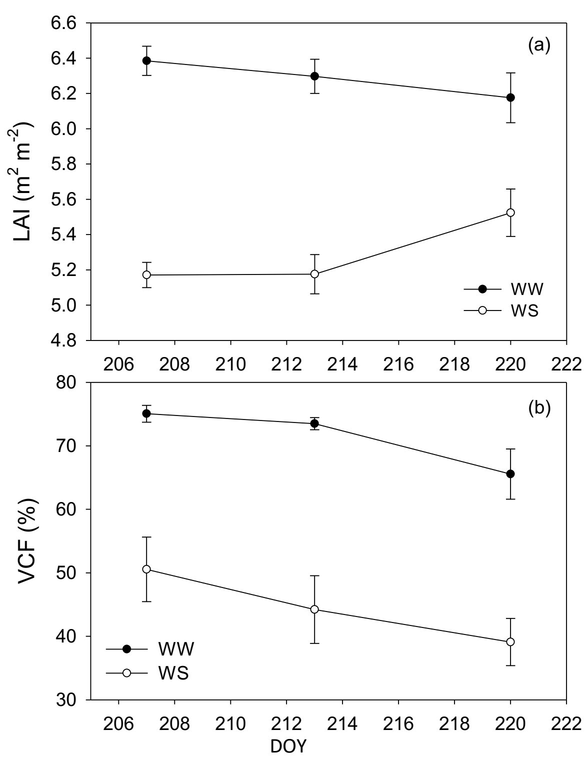

Fig. 8. (a) Leaf area index (LAI) and the (b) vegetal cover fraction (VCF) for (WW and WS) treatments through time. Points represent the mean value of the six cultivars analyzed. The vertical bars represent the mean standard error (n=6). LAI and VCF were estimated from hemispherical images taken in the morning (8:00-9:00 am) and were analyzed with CAN-EYE.

Significant differences in LAI and VCF values between cultivars were not observed from (DOY: 207 to 213) for LAI and VCF values (Fig. 9). However, on DOY: 220, significant differences were observed due to the beginning of the senescent phase beginning. Respect For instance, to the LAI, cultivar 16 showed a significantly value lower LAI than cultivars 4, 8 and 14, cultivars, while cultivar 6 showed an VCF value statically significantly lower than all other in contrast all cultivars, with the 16 cultivar exception of cultivar 16. The differences observed in LAI and VCF between cultivars in LAI and VCF do not let explain the yield differences in the WW treatments. This fact is due to because the maize yield response is caused by a multigenic phenotype that multigenic which depends on physiological and physiology variables (Araus et al., 2008).

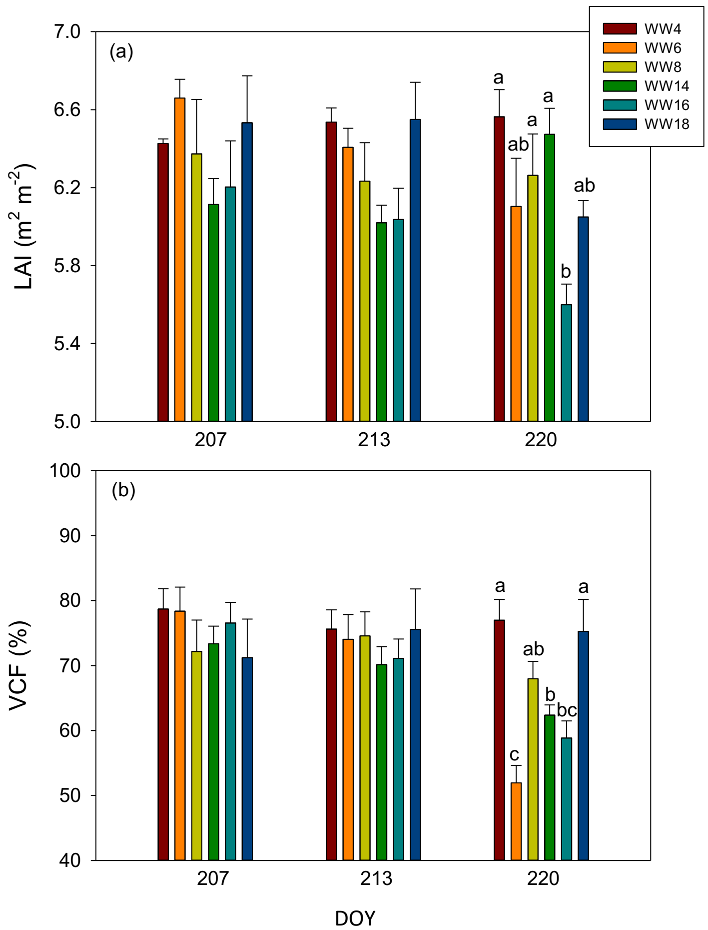

Fig. 9. Leaf area index (LAI) (a) and vegetal cover fraction (VCF) values (b) for the six cultivars during grain filling in the WS treatment. The vertical bars represent the mean standard error (n=3). Different letters indicate statistically significant differences as measured by the Duncan test (P < 0.05).

The WS cultivars showed more variability in LAI and VCF values than the WW treatments (Fig. 10). The LAI value was not affected in each cultivar until the last day (DOY: 220), when cultivar 4 showed values higher than the other cultivars, except cultivar 14. Differences in VCF values were evidence from the beginning, with being cultivar 4 having higher which values were higher.

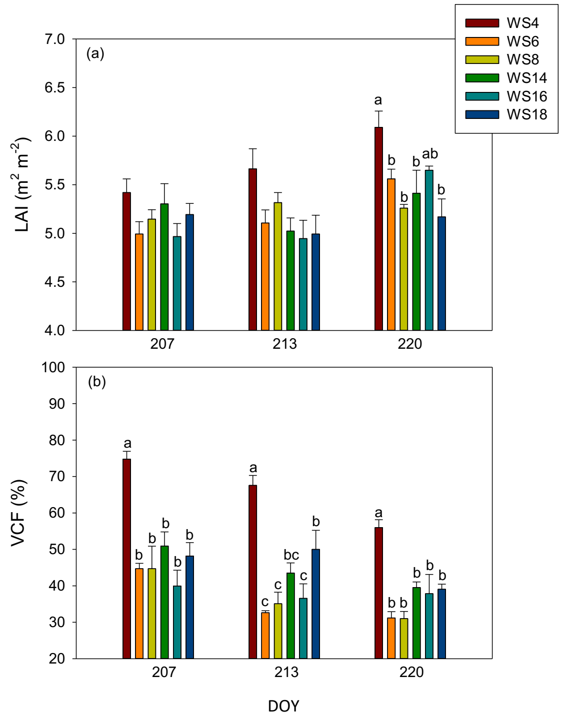

Fig. 10. Leaf area index (LAI) (a) and vegetal cover fraction (VCF) values (b) for the six cultivars during grain filling in the WS treatment. The vertical bars represent the mean standard error (n=3). Different letters indicate statistically significant differences as measured by the Duncan test ($P < 0.05$).

4. Conclusions

In a first approach, this method using bottom-up and top-down hemispheric images is a useful tool for the determination of the LAI and the VCF in maize plants.

The water regime imposed during the grain filling phase of the crop development cycle has a great impact on both LAI and VCF. The differences between the cultivars became apparent almost a month after the water deficit.

Comparing the measurements taken early in the morning and at noon under optimal irrigation conditions, the reductions in the LAI and VCF values were minimal, indicating that the varieties studied show a low foliar response to environmental stress. Under conditions of water stress, a strong reduction of VCF at noon was observed in all the cultivars tested. The reductions were much less evident in LAI, probably due to errors in estimating the agglomeration index under water stress conditions.

Considering the rapid improvement in drone technology for capturing high-resolution photos from above, in the future, other researchers can study the possibility of using new tools such as computer vision and artificial intelligence to estimate LAI. These technologies use software's such as Python and OpenCV in combination with neural networks to improve and automated image processing.

Acknowledgments

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