Conference Proceedings Paper

Comparison of Proximal Remote Sensing Devices for Estimating Physiological Responses of Eggplants to Root-Knot Nematodes

Alex Silva-Sánchez[1], Julia Buil-Salafranca[1], Andrea Casadesús Cabral[1], Naroa Uriz-Ezcaray[1], Helio Adán García-Mendívil[2], Francisco Javier Sorribas[1,2], José Luis Araus[1,3], Adrian Gracia-Romero[1,3]*

1 New Perspectives in Environmental Agrobiology, Master's degree in Environmental Agrobiology, Faculty of Biology, University of Barcelona, Barcelona
2 Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Esteve Terradas 8, 08860 Castelldefels, Barcelona, Spain
3 Integrative Crop Ecophysiology Group, Plant Physiology Section, Faculty of Biology, University of Barcelona, Barcelona, Spain

* Correspondence: adriangraciaromero@hotmail.com;

Published: date
Academic Editor: name

Abstract: Proximal remote sensing devices are becoming widely applied in field plant research to estimate biochemical (e.g. pigments or nitrogen) or agronomical (e.g. leaf area, biomass or yield) parameters as indicators of stress. Non-invasive characterization of plant responses allows screening larger populations faster than the conventional procedures that, besides being time-consuming, also imply the destruction of material or are subjective (e.g. visual ranking). This study deals with the comparison of a set of remote sensing devices at single-leaf and whole-canopy levels based on their performance in assessing how the eggplant and its yield respond to grafting as a way to tolerate root-knot nematodes. The results showed that whole-canopy measurements, as the Green Area (GA) derived from the RGB images (r=0.706 and p-value=0.001**) or the canopy temperature (r=-0.619 and p-value=0.005**), outperformed the single-leaf measurements, as the leaf chlorophyll content measured by the Dualex (r=0.422 and p-value=0.059) assessing yield. Moreover, other parameters as the time required to measure each sample or the cost of the sensors were taken into account in the discussion. Everything considered, indexes derived from the RGB images have demonstrated their robust potential for the assessment of crop status, being a low-cost alternative to other more expensive and time-consuming devices.

Keywords: Proximal Remote Sensing; Eggplants; Toot-Knot Nematodes; Single-leaf Measurements; Canopy Measurements; RGB images; Chlorophyll content; Leaf Temperature

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1. Introduction

The formulation of remote sensing indexes derived from multispectral information is extremely useful to assess plant diseases, detect stress or predict crop production [1]. Remote sensing techniques are used to monitor and phenotyping of cultivars properly, for instance, assessing biomass, water and nutrient status, or pigment content, which allows to practice precision agriculture. Traditional procedures in plant physiology studies are destructive (i.e. require the harvest of leaves or even the whole plant) and especially time-consuming [2]. The main benefits of using proximal remote sensing sensors are that measurements are done in-field conditions precisely, fast and without the need of destruct plant material [3]. There are several types of sensors based on the spectral reflectance and transmittance of the plants and they can work at leaf scale, as the clip-leaf sensors which optically measures the relative chlorophyll concentration [4], or at canopy scale, as the Normalized Difference Vegetation Index (NDVI) that is used for measuring biomass estimations [5]. Another approximation would be the canopy temperature measurements that are used for the detection of changes in stomata conductance as a response of water status [6]. However, the acquisition of that kind of devices entails notable economic costs. Contrary, information derived from RGB images is presented as an accessible low-cost alternative [7], proving agricultural farmers tools to predict production and adjust crop needs to crop supplies, allowing them to be precise in their crop management. Moreover, efforts are focused on the development of inexpensive instruments as the MultispeQ, a low-cost device that in addition to pigment content it provides measures of photosynthetic parameters [8]. Eggplant *(Solanum melongena*) has been receiving increasing attention the last decades regarding its phytochemical and nutraceutical components [9], but its production is threatened by root-knot nematode (*Meloidogyne javanica*). Root-knot nematodes are the most important agricultural pest in a large number of tropical and subtropical countries, where eggplant is widely cultivated [10]. It causes several root damages, restricting nutrient and water uptake of crops, and consequently, limiting the production. A proposed strategy to minimize the nematodes incidence on plant growth and yield, is to use resistant and tolerant rootstocks for grafting the eggplants. In this sense *Solanum torvum* may represent an adequate choice as rootstock. The aim of present study was to compare different proximal remote sensing approaches at plant and single leaf to assess the effect in eggplant of grafting with the tolerant species *Solanum torvum*. These remote sensing approaches evaluated water status and plant biomass of eggplants grafted in *Solanum torvum*. The ability to assess differences in the response to the growing conditions and to correlate to final yield was compared and discussed in order to conclude which proximal sensing approach (multispectral versus RGB-based indices) and at which level (leaf vs. whole plant) is the more convenient to assess how much ungrafted and grafted eggplant onto *S. toryum* are stressed by root-knot nematodes. Price of the sensors and the sampling procedures required were also compared.

2. Experiments

2.1. Plant material, growing conditions and data collection

The experiment was developed in a plastic greenhouse located at the experimental station of Agròpolis (41°17'18.1"N 2°02'38.5"E + 18 m a.s.l., approx..) of the Escola Superior d'Agricultura of the Universitat Politècnica de Catalunya (ESAB -UPC), in the municipality of Viladecans (Barcelona, Spain) from March to November 2018. Soil was artificially infested with *Meloidogyne incognita* in 2014 and cultivated with eggplant, lettuce, melon, tomato and watermelon the following years. A total of eighty eggplants, forty ungrafted and forty grafted onto the *S. torvum* cv. Brutus were transplanted in twenty plots, four plants per plot, in the same ones where were grown the previous year in order to know the putative selection of virulent nematode populations after the repeated cultivation of the resistant eggplant rootstock. The nematode population densities in soil at transplanting (Pi) the ungrafted eggplant ranged from 330 to 7584 juveniles 250 cm^{-3} of soil, and between 8 and 1292 juveniles 250 cm^{-3} of soil in plots transplanted with the grafted one. Each plot consisted of four plants spaced 0.55 cm each growing under a plastic greenhouse and fertilized by a drip irrigation system. Eggplants were harvested and weighed when fruits reached the commercial standards.

Remote sensing evaluations were performed on 1st October 2018 during the practical lessons of the subject of New Perspectives in Environmental Agrobiology of the Master's degree in Environmental Agrobiology. The measurements were divided as leaf-based and plant -based assessments and both were conducted from 15:00 to 17:00h. Measurements at single-leaf level were performed using two different clip sensors. On the one hand, leaf pigment contents were assessed using Dualex leaf-clip portable sensor (Dualex Force-A, Orsay, France), that measures chlorophyll

(Chl), flavonoid (Flav) and anthocyanin (Anth) content [11]. In addition, it calculates the nitrogen balance index (NBI), which is the ratio Chl/Flav related to the nitrogen and carbon allocation [12]. On the other, the MultispeQ (Michigan State University, Michigan, USA) device controlled by the PhotosynQ platform software [8] is an instrument that combines the functionality of a handheld fluorimeter, a chlorophyll meter, and a bench-top spectrometer. Moreover, it includes sensors of abiotic factors as the ambient temperature, relative humidity, barometric pressure and altitude as well as contactless leaf temperature, leaf angle and leaf direction. Among all the parameters that estimates, we used the measures fluorescence base parameters as the quantum yield of photosystem II (PSII) photochemistry (Φ_{II}), the quantum yield of non-photochemical quenching (Φ_{NPQ}) or the quantum yield of other unregulated (non-photochemical) losses (Φ_{NO}), and the relative chlorophyll content (Rel Chl). One RGB picture was taken per plot, holding the camera at 80 cm above the plant canopy in a zenithal plane and focusing near the centre of each plot. The conventional digital camera used was a Lumix GX7 (Panasonic, Osaka, Japan), a digital single lens mirrorless camera with an image sensor size of 17.3 × 13.0mm. Images were taken at 16-megapixel resolution using a 20 mm focal length. The images were saved in JPEG format with a resolution of 4592 × 3448 pixels and were subsequently analysed using the MosaicTool plugin. This software includes a JAVA8 version of Breedpix 2.0 (https://bio-protocol.org/e1488, IRTA, Lleida, Spain) that enables the extraction of RGB indexes in relation to different colour properties of potential interest [7].The NDVI was determined at ground level using a portable spectrometer (GreenSeeker handheld crop sensor, Trimble, Sunnyvale, CA, USA) by passing the sensor over the middle of each plot at a constant height of 0.5 m above and perpendicular to the canopy. Canopy temperature (CT) was measured using a PhotoTempTM MXSTM TD infrared thermometer (Raytek, Santa Cruz, USA), pointing towards the canopy at a distance of 0.5 m in the opposite direction of the sun. Finally, combined RGB and thermal images were taken using the phone CATS60 (Caterpillar Inc., Deerfield, Illinois, U.S.) and processed with the CeralsMobile with Hue Enhanced Agricultural Temperature (H.E.A.T.) software (https://integrativecropecophysiology.com/software-development/cerealsmobile/, University of Barcelona, Spain).

3. Results and discussion

Grafting did not report any significant differences in the leaf-based measurement, but in contrast, a significant effect was found between treatments (ungrafted and grafted) for almost all the whole plant parameters, excepting those derived from the thermal camera of the mobile phone (Table 1). The *Meloidogyne* nematode damages directly the roots due its parasitic activity, causing a negative effect on the absorption of water and nutrient that is exposed as noticeable reduction in growing [13]. Root-knot nematodes did not affect directly the pigment composition of the leaf, as noted with the leaf-based measurements. Nevertheless, as nematode affects roots we expected a sharper nutritional deficit captured by the leaf-based measurements. Chlorophyll readings on the non-grafted plants were slightly lower than the grafted plants (data not shown). Still, a little impact was noticed in the correlations of the chlorophyll readings and some of the photosynthesis parameters against yield. On one side, chlorophyll readings are a useful screening criterion to detect stress associated effects [14]. Both leaf-clip devices (Dualex and Photosynq) use the same wavelengths to assess chlorophylls (650 and 940 nm). Apart from chlorophylls, Dualex also measures other pigments but no treatment effects were found for these other pigments moreover to don't correlate with yield. Photosynq provides information about photosystems status [8] and the fates of light energy absorbed [15], as the Φ_{II}, the Φ_{NPQ} or the Φ_{NO}. The combination of those traits could permit a wider assessment of the plant status. The main reason of the lack of statistically differences between grafted and ungrafted eggplants might be related to the fact that measurements were took too late in the phenological cycle of the eggplants, therefore, the only significant differences reported were in growth. In fact, relative changes in yield of grafted and ungrafted cucumber were related to the relative variation in both R672 / [R550 x R708] and net photosynthetic rate [16], confirming previous results in which relative dry top weight of cucumber [17] or zucchini-squash biomass [18] was related to relative leaf chlorophyll content measured earlier in the plant-nematode interaction and in situations in which root-knot nematode is the main biotic stressful agent. Alternatively, the effect of nematodes in the plant status was mild, but accumulative. Moreover, the operator subjectivity in choosing the leaves to be clipped can represent a cause of error. Finally, the major drawback of the leaf-based clip sensors is the time required to take the measurements. This time-consuming limit their application in large scale studies, since an elevated number of replicates is needed to take representative measures of the whole plot variability. Comparing both devices, the sampling with the Photosynq is even more complex as it needs to be operated through Bluetooth using a smartphone and takes longer to measure on single leaf. However, in terms of price, Photosynq is much cheaper than the Dualex.

Table 1. Summary of the different sensor/techniques used to assess crop status. Each technique was evaluated in terms of accuracy, sampling difficulty, sampling time, post-processing and cost. Colour intensities correspond to higher or lower evaluation results. Significant differences between grafted and non-grafted plants were tested by one-way ANOVA. ns, not significant differences. R, correlation with yield

Level	Sensor	Trait	Accuracy		Sampling difficulty	Sampling time	Post-processing	Cost
			ANOVA	R				
Leaf-based	Dualex	Chl	ns	0.422	■□□□□	■■■□□	■□□□□	■■■■■
		Anth	ns	0.105				
		Flav	ns	-0.270				
		NBI	ns	0.277				
	Photosynq	Phi2	ns	0.438	■■■□□	■■■■■	■□□□□	■■■□□
		PhiNO	ns	-0.169				
		PhiNPQ	ns	-0.388				
		Rel Chl	ns	0.526				
		Fv'/Fm'	ns	0.335				
Canopy-based	GreenSeeker	NDVI	*	0.601	■□□□□	■□□□□	■□□□□	■■■□□
	RGB images	Hue	*	0.662	■■□□□	■□□□□	■■■□□	■□□□□
		GA	**	0.706				
		GGA	*	0.635				
		NGRDI	**	0.642				
	Infrared gun	CT	**	-0.618	■□□□□	■□□□□	■□□□□	■■□□□
	Thermal camera + RGB	Hue	**	0.590	■■□□□	■□□□□	■■■□□	■■■□□
		GA	*	0.472				
		GGA	**	0.547				
		CT	ns	-0.157				
		CT[GA]	ns	-0.154				
		CT[GGA]	ns	-0.154				

On the other side, canopy-based measurements are presented as a better alternative to assess the nematode resistance. The use of RGB indexes derived from conventional digital cameras is being widely applied for monitoring crop growth and crop status [19–21]. By the assessment of the colour tonalities of the image with the Hue parameter, the percentage of green pixels can be calculated [7]. GA is the relative amount (in per one) percentage of pixels in the image with a Hue range from 60º to 180º, including yellow to bluish green colour values. Meanwhile, GGA is more restrictive, because it reduces the range from 80º to 180º, thus excluding the yellowish-green tones. The formulation of GA (p-value < 0.001, R=0.706) improved the crop assessment with the Hue parameter itself (p-value < 0.05, R=0.662) and the more restrictive index GGA (p-value < 0.05, R=0.635). The GA is a reliable estimation of the crop coverage of the soil. The fact that the eggplants were in a late growing stage, an elevated percentage of leaves were senescent. At this moment, was more important to assess differences in growth rather than the plants that were staying green longer. Yet, RGB indexes derived from the smartphone camera followed a different trend: the Hue values (p-value < 0.001, R=0.590) outperformed GA (p-value < 0.05, R=0.472) and GGA (p-value < 0.001, R=0.547) formulations. That might be due the differences in image resolutions of both devices (digital camera vs. phone camera). From the RGB images was also calculated the NGRDI, which is an index similar to NDVI but uses information from the green instead of the near-infrared bands. The NDVI is one of the most well-known vegetation indexes to assess crop biomass. However, our results showed than even if the Red-Green reflectance break is smaller than the Red-NIR break, the NGRDI (p-value < 0.001, R=0.642) outperformed the NDVI (p-value < 0.05, R=0.601). These results support the indexes derived from conventional digital images as efficient and low-cost alternatives to the specialized and more expensive sensors like the GreenSeeker. Finally, the canopy temperature (p-value < 0.001, R=-0.618) was also assessed and it was reported as an accurate parameter for determining the crop resistance to the nematode. Decreases in stomatal conductance and transpiration rates as response to water stress cause an increases in plant temperature [22]. Nevertheless, the temperature measurements from the thermal camera of the CATS60 phone failed (p-value > 0.05, R=-0.157). The expected results were the opposite, as the CerealApp permits combining a GA and a GGA mask to the thermal camera in order to get an accurate assessment of the temperature of the green area, avoiding then noise from the soil that might affect the measurements with the infrared gun.

4. Conclusions

Single-leaf measurements did not show significant differences between grafted and non-grafted plants and their correlations with yield were generally low. Besides, plant-based measurements showed significant differences between both types of plants and higher yield correlations with yield. These findings suggest that plant-based measurements were more effective assessing the eggplants response to root-knot nematodes. Root-knot nematodes did not affect leaf chlorophylls. Even not being high-technology, RGB indexes reported significant differences between the growing treatments, moreover of showing the best correlations with yield. Plant temperature measurements with the infrared thermometer also performed well assessing differences in of plant resistance to the nematodes. It correlated negatively with yield, probably due that the roots less affected by nematodes had a better access to water and then stomata conductance was higher. However, when both categories of remote sensing traits (RGB indexes and canopy temperature) were measured simultaneously with the same device (smartphone), the results obtained were worse. This can be attributable to the fact that this measurement was taken later and the solar conditions changed. This clearly illustrates the importance of how and when (during the daytime) the temperature measurements are taken. To sum up, in terms of measurements at leaf level both Dualex and Photosynq are two interesting devices to study crop due to their high throughput measuring pigment and photosynthetic data and probably results could have been better if measured in an earlier phenological stage. The main drawbacks of these devices are due their time-consuming nature. Furthermore, canopy-based measurements permit to study the whole plot at once (without the need of replicates) and showed the best results. RGB indexes are presented as a promising remote sensing technique mainly due to its user friendly and low-cost nature. It should be noted that this measurement can be easily taken with a simple smartphone.

Acknowledgments: This work was supported by projects AGL2013-49040-C2-1-R, and AGL2017-89785-R funded by MINECO and FEDER. Thanks are given to CONACYT for providing PhD funding to H. A. García-Mendívil, to Dr. Shawn C. Kefauver (University of Barcelona) for providing the Cereal Scanner app and the personnel from Agròpolis.

Author Contributions: H.A.G.-M. and F.J.S. managed and directed the trial at the experimental station of Agròpolis. J.L.A. and A.G.-R.conceived and designed the experiment. A.S.-S., J.B.-S., A.C.C. and N.U.-E. conducted the field measurements and analysed the data. A.S.-S., J.B.-S., A.C.C., N.U.-E. and AG-R wrote the manuscript, under the supervision of J.LA. and F.J.S.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

RGB: Red-Green-Blue; GA: Green Area; GGA: Greener Area; NDVI: Normalized Difference Vegetation Index; ESAB-UPC: Escola Superior d'Agricultura of the Universitat Politècnica de Catalunya; Φ_{II}: Quantum yield of photosystem II (PSII) photochemistry; Φ_{NPQ}: Quantum yield of non-photochemical quenching; Φ_{NO}: Quantum yield of other unregulated (non-photochemical) losses; Rel Chl: Relative chlorophyll content

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