IECAG 2021

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Proceedings

Comparison of Proximal Remote Sensing Devices of Vegetable Crops to Determine the Role of Grafting in Plant Resistance to *Meloidogyne incognita* †

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† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://iecag2021.sciforum.net/.

Abstract: Proximal remote sensing devices are novel tools that enable the study of plant health status through the measurement of specific characteristics, including the color or spectrum of light reflected or transmitted by the leaves or the canopy. Among these, RGB images can provide spatially detailed information about crop status including estimates of biomass, chlorophyll (and chlorosis) and fractional vegetation cover. The aim of this study is to compare the RGB data collected during five years (2016–2020) of four fruiting vegetables (melon, tomato, eggplant and peppers) with trial treatments of non-grafted and grafted onto resistant rootstocks cultivated in a *Meloidogyne incognita* (a root-knot nematode, RKN) infested soil in a greenhouse. The proximal remote sensing of plant health status data collected were divided into three levels. Firstly, leaf level pigments were measured using two different handheld sensors (SPAD and Dualex). Secondly, canopy vigor and biomass were assessed using vegetation indices derived from RGB images and the Normalized Difference Vegetation Index (NDVI) measured with a portable spectroradiometer (Greenseeker). Thirdly, we assessed plant level water stress, as a consequence of the root damage by nematodes, directly using stomatal conductance measured with a porometer, and indirectly using plant temperature with an infrared thermometer and also the stable carbon and nitrogen isotope composition of leaf dry matter. Among the measured parameters, carbon and nitrogen percentage exhibited the highest positive correlation ($r = 0.9$), whereas flavonoids and NBI (Nitrogen Balance Index) showed the highest inverse correlation ($r = -0.87$). It was found that the interaction between treatments and crops (ANOVA) was statistically different for only 4 of 17 parameters (flavonoid ($p = 0.002$), NBI ($p = 0.044$), NDVI ($p = 0.004$) and CSI (RGB-based Crop Senescence Index) ($p = 0.002$). Concerning the effect of treatments across all crops, differences existed only in two parameters, which were flavonoid ($p = 0.003$) and CSI ($p = 0.001$). Grafted plants contained less flavonoids ($\bar{x} = 1.37$) and showed lower CSI ($\bar{x} = 11.65$) than non-grafted plants ($\bar{x} = 1.98$ and $\bar{x} = 17.28$, respectively, $p = 0.020$ and $p = 0.029$) when combining all five years and four crops. We conclude that the grafted plants were less stressed and more protected against nematode attack. Leaf flavonoids and the RGB indexes (CSI) were robust indicators of root-knot nematode impacts across multiple crop types.

Keywords: Proximal remote sensing; root-knot nematode; RGB images; rootstock; melon; pepper; eggplant; tomato

Citation: Hamdane, Y.; Gracia-Romero, A.; Buchaillot, L.; Sanchez-Bragado, R.; Fullana, A.M.; Sorribas, F.J.; Araus, J.L.; Kefauver, S.C. Comparison of Proximal Remote Sensing Devices of Vegetable Crops to Determine the Role of Grafting in Plant Resistance to *Meloidogyne incognita*. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Academic Editor:

Published: date

1. Introduction

Root-knot nematodes (RKNs) are responsible for significant economic losses to a wide variety of crops world-wide (Koenning et al., 1999). PPNs cause a reduction in crop yield by the direct destruction of root cells, or, indirectly by propagating viruses, or by facilitating the invasion of fungi and bacteria through lesions caused during their penetration into the roots. New techniques have been integrated in agriculture by advancements in precision agriculture and plant phenotyping that allow for rapid and non-destructive assessments of crop health (Koenning et al., 1999). To better study crop physiological status and nutrient or other management requirements, we should consider more effective and efficient measures such leaf sensors, proximal or remote sensing instruments. One of these techniques, RGB images, can provide information on the plant nutrient state and general health. This is interpreted from the analysis of RGB images, which effectively capture the whole range of photosynthetically active radiation (PAR) (Gracia-Romero et al., 2019). Hence, RGB image analysis techniques constitute a way for the detailed study of plants with non-destructive instruments.

In order to better assess the capacity of RGB proximal imaging analyses for making recommendations for improving crop productivity, we present here a comparison of RGB indexes to other scientific plant health sensors in the context of greenhouse horticultural crop production and the common pest of root-knot nematodes. We present a comparison of different RGB images collected from different species (melon, tomato, eggplant, pepper) and treatments (grafted, non-grafted) to highlight in the first place the technical capacity of these instruments as non-destructive tools to detect the different details about plant condition and in second place to confirm the role of agronomic techniques like grafting in the protection of plants against RKNs and for ensuring favorable growth and productivity.

2. Materials and Methods

2.1. Study Site

The research was carried out between 28 September and 8 October over five successive years from 2016 to 2020 in a semi-open greenhouse located at the experimental station of Agròpolis (of the Escola Superior d'Agricultura of the Universitat Politècnica de Catalunya (ESAB—UPC), in the municipality of Viladecans (Barcelona, Spain). Management of crops was maintained by the technicians of the Agròpolis experimental station following local fertilizer, irrigation and pollination standards designed to ensure favorable growth and productivity apart from the RKNs and root stock study treatments.

2.2. Plant Material and Trial Design

Over the five years of study, we rotated between four crops with half of the cultivation non-grafted and half of the plants grafted to nematode resistant root stock. In 2016, melon (*Cucumis melo*) was grafted with resistant root stock of melon (*Cv paloma*) with a total of 40 plots of melon planted, where each plot composed by five plants. For 2017, two crops were cultivated, melon (*Cucumis melo*) and tomato (*Solanum lycopersicum*), grafted with resistant root stock of melon (*Cucumis metuliferus*) and tomato (*Durinta* spp.). During this year the same distribution of 2016 was repeated, but two species, tomato and melon, and 80 plots planted and of, 40 of tomato and melon each. In the 2018 experiment, eggplant (*Solanum melongena*) was grafted with root stock of resistant melon (*Cucumis metuliferus*), with 20 samples of pepper were divided in four blocks, each one containing five plants. In 2019, peppers (*Capsicum annuum*) were grafted to RKN resistant root stock of pepper (*Capsicum annuum PI 201234*). Finally, in 2020 we cultivated tomato (*Solanum lycopersicum*) grafted with resistant tomato (*Durinta* spp.), was composed of 40 plots of tomato divided in plots containing five plants each.

2.3. Measurements and Sampling Methods

For the measurement of pigments, we used two handheld sensors (SPAD 502 and Dualex). The first tool determines the relative chlorophyll concentration by measuring the leaf absorbance in red and near-infrared regions (Spectrum Technologies Inc., Plainfield, IL, USA) (Gracia-Romero et al., 2019). Where the second measures the chlorophyll content of leaves using a transmittance ratio at two different wavelengths. The Dualex (Dualex, Orsay, France) furthermore also measures leaf flavonoid and anthocyanin content by a ratio of chlorophyll fluorescence and also the chlorophyll to polyphenol ratio, known as the nitrogen balance index (NBI) (Li et al. 2013).

In order to inform about plant health and vigor some canopy level parameters were also measured, where firstly we used the Trimble Greenseeker handheld crop sensor (Trimble, Sunnyvale, CA, USA) which provides Normalized Differenced Vegetation Index (NDVI) values. Then, performed the analyses of RGB images taken by Panasonic Lumix DMC-GX7 (Panasonic, Osaka, Japan) 16 MP camera in order to detect the percentages of yellow-green and green pixels as Green Area (GA), Green Greener Area (GGA), Normalized Green Red Difference Index (NGRDI), Triangular Greenness Index (TGI), and Crop Senescence Index (CSI), calculated using the CerealScanner plugin for FIJI (http://gitlab.com/sckefauver/cerealscanner, Fiji is just ImageJ, http://fiji.net). Below are the equations of the different parameters, where R represents the reflectance at the indicated approximate wavelength (Equations (1)–(3)).

$$TGI = -0.5\ [190(R670 - R550) - 120(R670 - R480)] \text{ (Hunt el al., 2011)} \quad (1)$$

$$NGRDI = (R550 - R670)/(R550 + R670) \text{ (Hunt et al., 2012)} \quad (2)$$

$$CSI = 100 \times (GA - GGA)/GA \text{ (Vergara-Diaz et al., 2015)} \quad (3)$$

Measuring stomatal conductance using porometer (Decagon Leaf Porometer SC – 1) (Montague et al., 2008), plant temperature measured by Raytek PhotoTemp TM XMXSTM TD infrared thermometer (Raytek, Santa Cruz, CA USA, Duncan et al., 2005) and analysis of both C and N stable isotopes in leaf dry matter. Briefly, the scientific protocol followed was that dry leaves were ground to a fine powder and 0.7–0.9 mg of leaf dry matter from each plot was weighed and sealed into tin capsules and sent for analyses. Stable carbon (13C/12C) and nitrogen (15N/14N) isotope ratios as well as the leaf N and C concentrations (%) were measured using an elemental analyzer (Flash 1112 EA; Thermo Finnigan, Bremen, Germany) coupled with an isotope ratio mass spectrometer (Delta C IRMS, Thermo Finnigan) operating in a continuous flow mode. Samples were loaded into a sampler and analyzed by technical services staff. From these measurements we assessed the level water stress experienced by the plant and indirectly the root health (see Vergara-Díaz et al., 2016 for more details).

2.4. Statistical Processing

Statistical treatment was done using Statgraphics Centurion XVI (Developed by Statpoint Technologies, Warrenton, VA, USA) for basic data analyses like mean and standard error and ANOVA. For correlation between different was done by MS Office Excel 2007 (developed by Microsoft, Redmond, WA, USA). Finally, the graphics was obtained using Sigma Plot 12.5 (Statistical software developed by Systat software, Chicago, IL, USA).

3. Results

In preliminary data exploration, it was found that the correlation percentage C and percentage N recorded the highest value of direct correlation ($r = 0.9$). In contrast, the highest inverse correlation is recorded for correlation between flavonoid and NBI ($r = -0.87$). This means that the percentage carbon was positively affected by the production of nitrogen in the plant It is known that the carbon and nitrogen are the two important elements in the construction of plant structure and both appear to be affected by PPN damage. In the example of flavonoids and NBI, for the second inverse correlation if the value of one increases the value of the second decreases. We note that amount of nitrogen in the plant decreased with an increase of flavonoid production, which understood that flavonoid as a hormonal regulator that aides in the plant adaptation to stress, which in this instance is inversely related to the nitrogen balance and suggestive of both N and C limitations to uptake by the RKNs.

Table 1. ANOVA, mean and standard deviation of three levels of parameters (leaf level pigments, canopy vigor and biomass, water stress and root health) achieved for four crops (melon, tomato, eggplant, pepper) with two treatmentsV (grafted and non-grafted) n = 152 during five years (2016–2020) in a greenhouse in soil infected by root-knot nematode. We also show the effect separately of crop treatments and the interaction of the both. SPAD (Soil Plant Analysis Development), Chl (chlorophyll), Flav (flavonoid), Anth (anthocyanin), NBI (Nitrogen Balance Index), GA (Green Area), GGA (Greener Green Area), TGI (Triangular Greenness Index), NGRDI (Normalized Green Red Difference Index), CSI (Crop Senescence Index), δ13C (isotopic composition of carbon 13), percent C (percentage of carbon), δ15N (isotopic composition of nitrogen 15), percent N (percentage of nitrogen), Normalized Difference Vegetation Index (NDVI), and porometer stomatal conductance (gs).

	Parameters	***p* Value Treatments**	***p* Value Interaction Crop*Treatments**	**Mean Grafted**	**SE Grafted**	**Mean Non-Grafted**	**SE Non-Grafted**
Leaf level pigments (SPAD and multiple parameters from the Dualex)	SPAD	0.110	0.380	45.77	0.87	43.82	0.86
	Chl	0.140	0.066	26.03	0.50	27.07	0.53
	Flav	0.003	0.002	0.91	0.04	1.00	0.02
	Anth	0.607	0.565	0.06	0.01	0.07	0.02
	NBI	0.060	0.044	33.66	0.64	32.02	0.61
Canopy vigor, biomass from yellow to green and green fractional vegetation cover (GA and GGA from RGB images)	GreenSeeker NDVI	0.768	0.004	0.46	0.03	0.46	0.02
	GA	0.725	0.606	0.39	0.02	0.38	0.03
	GGA	0.102	0.810	0.29	0.03	0.34	0.02
	NGRDI	0.474	0.889	0.06	0.26	0.33	0.27
	TGI	0.322	0.810	4145.16	444.77	3516.36	449.86
	CSI	0.001	0.002	34.15	1.92	25.07	1.90
Water stress and root health	gs	0.724	0.267	114.24	5.35	111.57	5.34
	Temperature	0.178	0.654	25.62	0.20	25.24	0.22
	δ13C	0.819	0.984	−26.26	0.85	−25.99	0.82
	Percent C	0.702	0.925	30.35	1.01	30.89	0.98
	δ15N	0.335	0.121	5.61	0.23	5.30	0.22
	Percent N	0.793	0.210	2.36	0.10	2.33	0.09

From Table 1 firstly, for treatments the two factors are statically significant are Flav and CSI. While, for interaction crop*treatment adding to the two previous parameters are NBI and Greenseeker NDVI. The significant difference of all parameters for crop does not have importance considering that each species has physiological and biological characteristics different that greatly affect the all the measurement values and have thus been removed. We note also that grafted plants exceeded the non-grafted plant in most parameters such as SPAD, NBI, GA, TGI, CSI (δ13C, δ15N, percent N. Despite, non-grafted plant dominates some parameters like Chl, Flav, Anth, GGA, NGRDI, and percent C. It is seen from the comparison of grafted and non-grafted plant that the most important parameters that show the good functioning of the plant are in the favor of the grafted plants. We understand also from this comparison that non-grafted plant was stressed and suffers much from the RKN infestation.

4. Discussion

Grafting onto resistant-tolerant rootstocks is a promising non-chemical way to suppress RKN populations and to reduce yield losses of the most susceptible cucurbit crops. Similar results as those presented here in this study have been previously discussed by several authors, which touched on the topic in relation to similar species and the impact of nematodes on crop health. Plant resistance has been noted as an effective and profitable control method to reduce RKN reproduction rates and equilibrium density (Sorribas et al., 2005). Grafting was able increase the uptake of water and nutrients resulting in larger root systems and increased diseases tolerance (Miguel et al., 2004).

The internal transport mechanisms of the plants benefitted from the grafting, as indicated by the low δ13C and δ15N recorded in non- grafted plants as followed by a reduction in water-use efficiency; this may have other consequences, such as slowing down plant metabolism (Haverkort, & Valkenburg, 1992). The leaf surface area of the plant and photosynthetic capacity of the plant is approximated by the measurement of Greenseeker NDVI. However, according to the results obtained, the grafted plants recorded similar NDVI values as the non-grafted crops, indicating that these plants developed similar vigor, when considering across all crops, though the interaction indicates that the were difference for some crops. Similarly, Silva Sanchez et al. (Silva Sanchez et al., 2019) noted that no NDVI difference was detected between grafted and non-grafted eggplants. Though not presented here, productivity measures would be a more definitive estimate of the overall vigor, the combination of Chl and biomass measured by the Greenseeker NDVI, and total impacts of RKNs.

The flavonoid biosynthesis pathway in the plant can be induced by a broad pathogenesis response through jasmonic acid, salicylic acid, ethylene, and auxins, likely triggered when the RKNs cause mechanical damage and wounding during feeding and penetration (Goverse et Smant, 2014). The higher flavonoid levels recorded in non-grafted plants indicate plants under stress. If we know that N is a key elemental component in chlorophyll—the biomolecule that allows plants to absorb energy from light—this confirms the major role of this macro-elements in the vital processes of plants, and is supported by higher NBI values recorded by grafted plants. Higher NBI indicates that physiological process were more efficient and grafted plant were less affected by nematode attack. Also, NBI indicates higher photosynthesis activity, which is different to the study (Silva Sanchez et al., 2019) that did not detect any difference between grafted and non-grafted plant in NDVI.

Senescence may also be advanced by biotic or abiotic factors such as attacks by pathogens (e.g., nematodes in our case), and in our study was assessed by the value of CSI in non-grafted plants of some crops, as tomato, exceed that of grafted. Higher CSI senescence can be affected by light intensity, water availability, and nutrition, which all controlled in this study, so the most important here was the RKN impacts, which accelerated the process of senescence and by shortening the life cycle of the plants by reducing the final production quantity and also potentially the quality (Munné-Bosch et al., 2013). TGI was measured as an indicator of chlorophyll content, and thus, the nutrition state of the plant and functioning of photosynthesis processes in the plant. Higher values of TGI in grafted plants in some crop means that these plant contained more chlorophyll and that the attack of nematodes did not affect as much the production of plant pigments in grafted crops, highlighting the role of TGI as a useful an RGB index in crop health monitoring (Yang et al.,2020).

5. Conclusions

According to all the results obtained over the four successive years, we note that the grafting techniques constitute a means of protection against attack by RKNs. This is seen in the majority of cases studied by the value of physiological parameters (Greenseeker, NBI, Flav, CSI, TGI) of grafted plants which indicate better crop health than that of non-grafted plants. These limited results indicate good functioning of the plant physiological defense processes in the grafted plants inversely to that of the non-grafted, which suffered from the intensity of attacks by nematodes through the limited uptake of key resources and the increased activation of defense mechanisms. Grafting has been previously observed to be more effective on some crops and is potentially linked to the compatibility between the rootstock and graft and quality of benefits obtained from the rootstock. Some root stocks are more effective than others and bring more benefits to the plant, which affects the growth and the final production in addition to the resistance to this pest. Our study also supports the role of RGB images as a nondestructive and low-cost technique that ensures a detailed diagnosis of plant physiological status. RGB image analyses are being used more and more in agricultural studies on the one hand to save time but also to more effectively monitor crop status in order to ensure the best possible condition for development and subsequently optimize production. Taking into account climate change, resistant root grafting may reduce the damage caused by RKNs, but may also necessitate the development of new more effective rootstocks, adapted not only to the biotic soil factors but also to changing environmental conditions. New resistant root stock breeding efforts may be supported by genetic engineering to improve rootstocks with a more favorable adaptation to global change.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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