USE OF MULTICOLOUR FLUORESCENCE IMAGING IN PLANT PHENOTYPING

María Luisa Pérez-Bueno, Mónica Pineda and Matilde Barón

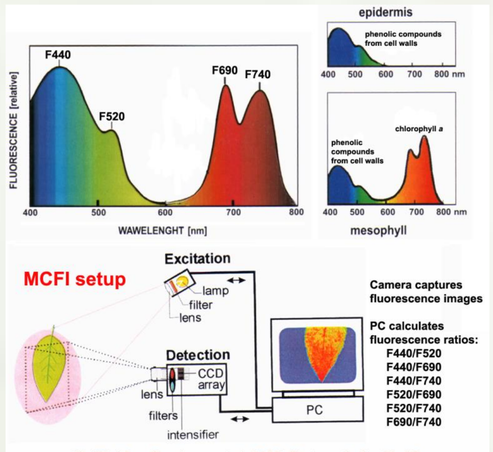
Department of Biochemistry and Molecular and Cellular Biology of Plants, Estación Experimental del Zaidín (CSIC), Granada, Spain

INTRODUCTION: MULTICOLOUR FLUORESCENCE IMAGING IN BASIC RESEARCH

Multicolour fluorescence imaging (MCFI) is based on the acquirement of fluorescence images of leaves upon excitation with long-wavelength UV radiation (1).

Phenolic compounds (such as ferulic or chlorogenic acids) usually increase upon stress as part of the plant defence response (2).

Fluorescence ratios are also informative (1, 2):
- Early stress indicators: F440/F690 and F440/F740
- Long stress exposure: F440/F520
- Chlorophyll content: F690/F740.

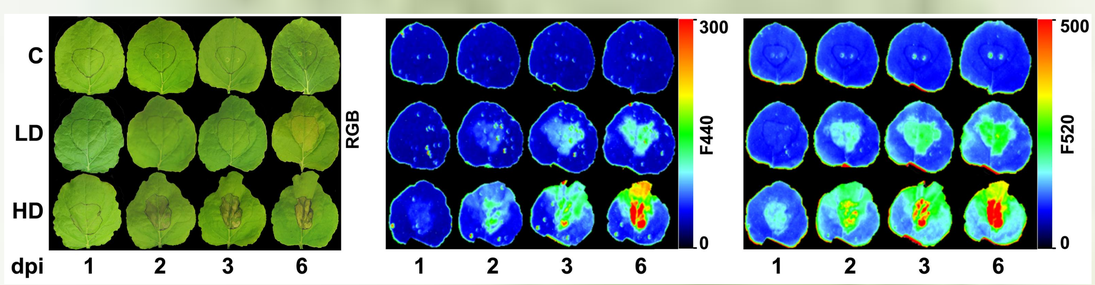


Modified from Buschmann et al. (2000) Photosynthetica 38: 483

MCFI could be a very useful tool to analyze stressed plants, identifying specific patterns that can be correlated with particular stress factors.

Advances have been made on the implementation of multicolour fluorescence to crop fields (8, 9). However, these non-imaging portable devices do not allow the study of crops at large scale. Thus, the development of MCFI devices designed for their use in greenhouses and crops would be a great step forward in the field of crop phenotyping.

MCFI has long been used as a very informative technique for fundamental research on plant defence responses upon stress (2). Some works have applied MCFI on crop fields, although no systematic analysis of the images could be carried out at that time.
- F440/F690 and F440/F740 are sensitive to several nutrient deficiencies for maize and sugar beet growth (3, 4), and to diuron-treated leaf parts (5, 6).
- F440 and F520 increase in the punctures produced by white tobacco flies (1).
- F440/F690 and F690/F740 are sensitive to mites attack (7).
- The model plant *Nicotiana benthamiana* showed increased values of F440 and F520 when infected with the necrotrophic bacterium *Dickeya dadantii* 3937, that were proportional to bacterial dose:



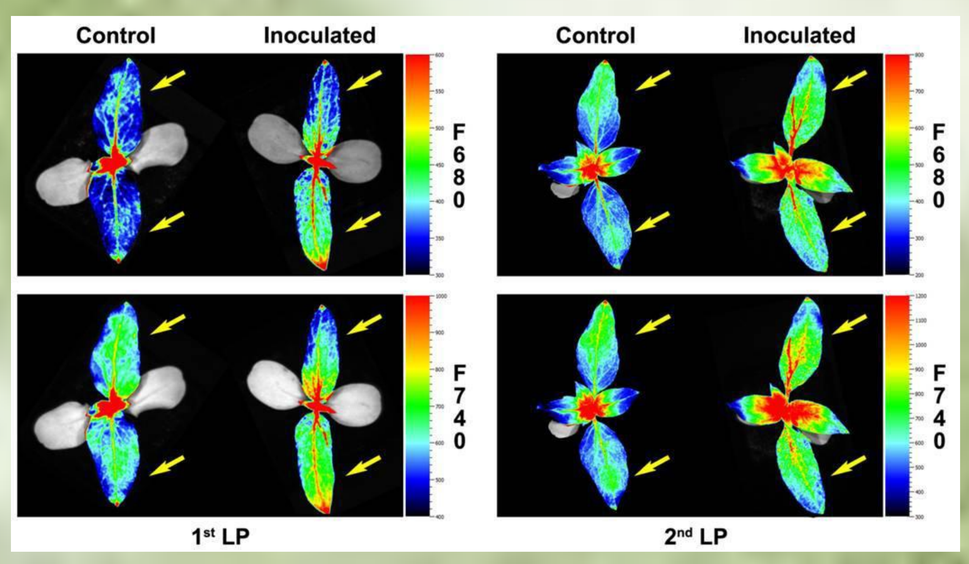
Images of *N. benthamiana* plants infected with the bacterium *D. dadantii* at low (LD) and high dose (HD).

Images taken from Pérez-Bueno et al. (2016) Front. Plant Sci. 6: 1209

LITERATURE CITED:

1. Buschmann and Lichtenthaler (1998) J. Plant Physiol. 152: 297
2. Cerovic et al. (1999) Agronomie. 19: 543
3. Heisel et al. (1996) J. Plant Physiol. 148: 622
4. Langsdorf et al. (2000) Photosynthetica 38: 539
5. Lichtenthaler et al. (1997) Bot. Acta 110: 158
6. Edner et al. (1995) EARSeL Advan. Remote Sens. 3: 14-2
7. Lang (1995) Karlsruhe Contrib. Plant Physiol. 29: 1
8. Tremblay et al. (2012) Agron. Sustain. Dev. 32: 451
9. Latouche et al. (2015) Photochem. Photobiol. Sci. 14: 1807

RESULTS: MULTICOLOUR FLUORESCENCE IMAGING APPLIED TO CROP SCIENCES

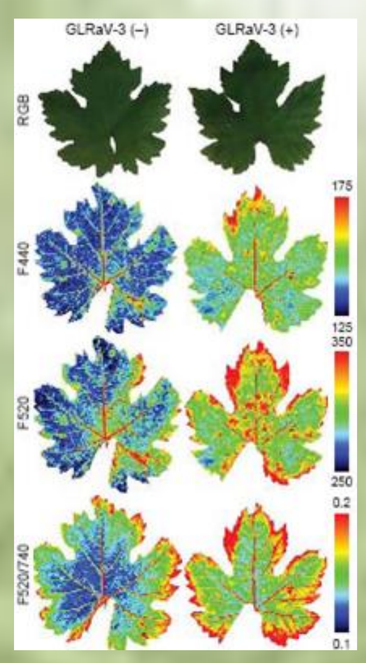


F680 and F740 images of the first and second leaf pairs (LP) of sunflower (*Helianthus annuus L.*) plants inoculated with broomrape (*Orobanche cumana*) and control plants.

Images taken from Ortíz-Bustos et al (2016) Front. Plant Sci. 7: 884

Broomrape is a root holoparasit causing no symptoms on the aerial part of sunflower plants prior to the emergence of the parasite. The parasitized plants could be detected by increases in F680 and F740 when they were only 2 weeks old (panels on the left).

This work was the first time that the impact of a root parasite on its host plant was examined by MCFI.

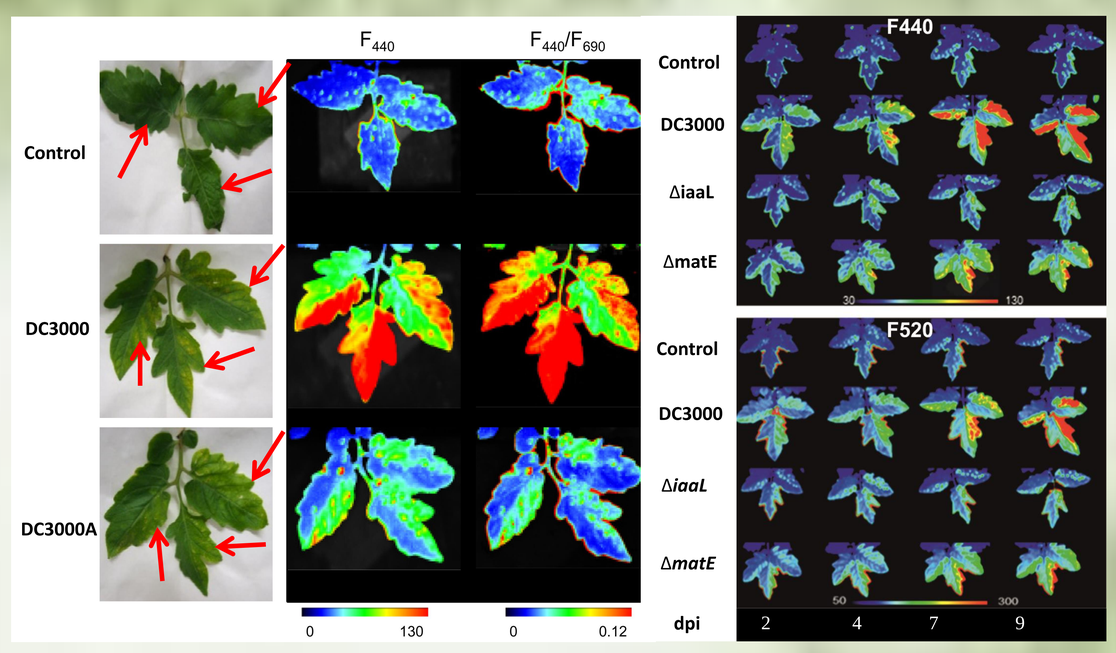


Images of F440, F520 and F520/F740, on grapevine (*Vitis vinifera* "Malvasía de Banyalbufar") leaves upon infection with Grapevine leafroll-associated virus 3, GLRaV-3 (+), and controls, GLRaV-3 (-).

Images taken from Montero et al. (2016) Physiol. Plant. 157: 442

GLRaV-3, a phloem-limited virus, is one of the most important viruses affecting grapevines. It infects both white and red grape varieties. In this white variety, symptoms are not so clear or even absent.

Higher concentrations of flavonols and hydroxycinnamic acids as a consequence of the viral infection can account for the increased values of F440, F520 and F520/F740 in the infected leaves.



Images of F440, F520 and F520/F680 from healthy tomato (*Solanum lycopersicum*) and infected leaves either with *Pseudomonas syringae* p. tomato DC3000 (Pto) or with its mutants: DC3000A, Δ*iaaL* and Δ*matE.* The right half of each leaflet (marked with arrows) was infiltrated with bacteria, or mock-infiltrated.

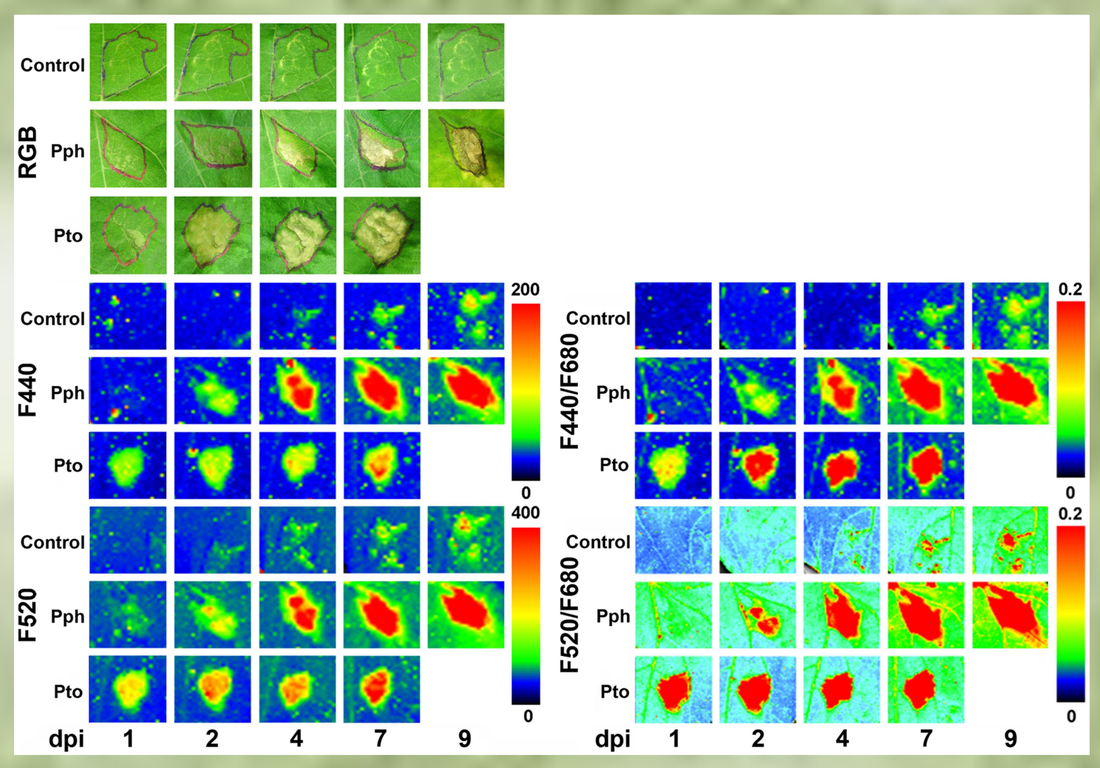
Images taken from Vargas et al (2013) Environ. Microbiol. Rep. 5: 841, and Castillo-Lizardo et al. (2015) BMC Microbiology 15: 165

Pto, an important model in molecular plant pathology, causes bacterial speck on tomato. DC3000A is a mutant lacking a multidrug resistance efflux pump, Δ*matE* lacks a transporter of the multidrug and toxic compund extrusion family. The *iaaL* gene, related to indole-3-acetic acid metabolism, is deleted In the Δ*iaaL* mutant.

The development of symptoms in Pto-plants infected with its different mutants was delayed and that was related to lower bacterial populations in infected tissues when compared to wild-type Pto-infected plants.

Plants inoculated with the pathogens exhibited larger values of F440, F520 and F520/F680 in inoculated areas than the corresponding control plants, related to the up-regulation of the plant secondary metabolism for defence. In the plants infected with the Pto mutants this activation and the corresponding MCFI values (lower that those induced by the Pto wild type) are proportional to the bacterial population and to the accumulation of flavonoids produced in response to these bacterial strains.

MCFI was used to determine the virulence of different bacterial mutants



Images of F440, F520, F440/F680 and F520/F680 from healthy bean (*Phaseolus vulgaris*) leaves, compared to plants infected with *Pseudomonas syringae* pv. phaseolicola 1448A (Pph) and pv. tomato DC3000 (Pto).

Taken from Pérez-Bueno et al. (2015) Physiol. Plantarum 153: 161

Pph and Pto are excellent models for fundamental studies on bacterial attack and plant defense. In bean plants, Pph is the causal agent of halo blight systemic disease, while Pto elicits hypersensitive response.

In the absence of symptoms, Pph and Pto-infected plants showed larger values of F440, F520, F440/F680 and F520/F680 in the inoculated areas than the respective controls. Tissues surrounding the Pph-inoculated areas also showed a rise in the value of these parameters, correlated with an increase on phenolic compounds. However, changes on MCFI in Pto-infected leaves was restricted to the infiltrated areas.

MCFI discriminated between a bacterial systemic infection and a hypersensitive response in the absence of symptoms

This work was supported by RECUPERA 2020 (20134R060, Ministerio de Economía y Competitividad-CSIC, ERDF) and Junta de Andalucía (P12-AGR370)