Scientific Report on the STSM '**Assessing the properties of the photosynthetic apparatus in grafted plants under abiotic stress by chlorophyll fluorescence imaging'**

Reference COST Action FA1204

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The purpose of this STSM was 1) to evaluate grafting compatibility degree by CFI using different rootstocks and scions and 2) to study the physiological responses in plants (eggplant, pepper and tomato) under water and salinity stress.

Here, I report on the STSM activities carried out during my stay at the host institution (IVIA). All activities were undertaken under supervision of Dr. Angeles Calatayud.

A- Protocol for Chlorophyll fluorescence imaging

The IMAGING-PAM Chlorophyll Fluorometer (Walz, Germany) was used to take measuring images of photosynthetic activity in grafted plants (**Fig. 1**). Chlorophyll fluorescence imaging (CFI) was used to study the degree of graft compatibility in different plant combinations: pepper/pepper, pepper/tomato and pepper/eggplant which were indicated as compatible, incompatible and optional compatible, respectively. In addition, CFI was used to test the photosynthetic damage on the leaves under water stress and salinity.

1- Chlorophyll fluorescence to determine compatibility and incompatibility in grafted plants.

Measurements of CFI were performed in the early stages of grafts (15 days after grafting). Behavior and changes of the different chlorophyll fluorescence variables in compatible and incompatible grafts were observed using ImagingWin software and analyzing the kinetics curves. The variables obtained from dark-adapted material were **Fo** (the minimum fluorescence signal), **Fm** (the maximum fluorescence signal), **Fv** (variable fluorescence; Fm - Fo) and **Fv/Fm** (maximal PSII quantum yield). From light-adapted material **F'o** (the minimum fluorescence signal), **F'm** (the maximum fluorescence signal), (F`m-Fs)/F`m (ΦPSII actual quantum efficiency) were assessed together with **NPQ** = (Fm-F`m)/F`m (quenching of variable fluorescence) and **qP** = (F`m-Fs)/(F`m-F`o) (photochemical quenching). The fluorescence imaging was obtained in the graft area, rootstock and scion (**Figs. 2-4**).

2- Chlorophyll fluorescence in salt and water stress.

Kinetics and images from the above CFI parameters were measured in independent leaves of pepper, tomato and eggplants under control conditions compared with abiotic stress treatments (**Figs 5-6**).

B- Physiological responses in plants under water and salinity stress

In addition, the effect of two different abiotic stresses (salinity and water stress) on different physiological parameters was studied in pepper, tomato and eggplant. Plants were grown under control conditions, 80 mM NaCl and with 50% water reduction to provoke salinity and water stress respectively. A series of physiological measurements have been programmed to see the differences comparing with control plants. Physiological parameters: lipid peroxidation, accumulation of proline, osmotic potential and chloride concentration (Cl^-) were measured in leaves.

The **osmotic potential** of leaf sap was measured with an osmometer (digital osmometer, Wescor, Logan, USA). The osmotic potential (ψ_s) was significantly reduced under salinity stress in all plant species (**Fig. 7**), and the same effect when compared to control plants was found for tomato plants under water stress.

The **chloride concentration** (Cl^-) in the dry plant material was extracted with 0.1 N HNO_3 in 10% (v/v) acetic acid and was determined by potentiometric titration with $AgNO_3$ in a chloride analyzer (Sherwood, MKII 926). The results were expressed as mmol g^{-1}DW. The Cl^- concentration in leaves was approximately 3 times higher under NaCl when compared to control plants (**Fig. 8**).

Proline content (mg g−1DW) was determined in leaf tissue (0.02 g) which was ground in 3% sulphosalicylic acid. The homogenate was filtered and 0.6 mL of glacial acetic acid and 0.7 mL of ninhydrin reagent (2.5 g ninhydrin in 600 mL glacial acetic acid and 40 mL 6 M phosphoric acid) were added to an aliquot of the filtrate. The reaction mixture was boiled for 1 h, and readings were taken at a wavelength of 520 nm in a spectrophotometer. Both stress conditions induced increase of proline content in leaves but significantly the highest concentrations were observed under salinity stress (**Fig. 9**).

Lipid peroxidation in leaves was estimated through malon-dialdehyde (MDA) determinations using the thiobarbituric acid reaction following the protocol reported by Heath et al. (1968), and modified in Dhindsa et val. (1981). The non-specific background absorbance reading at 600 nm was subtracted from the specific absorbance reading at 532 nm. MDA (nmol MDA g -1FW) content ranged from 12.9 to 17.8 in eggplant, from 9.6 to 13.4 in pepper and from 6.1 to 7.1 in tomato. MDA content did not increase under water and salinity stress (**Fig. 10**), probably due to a shorter term under these stresses or in this conditions there were not oxidative damage in the membrane lipid.

The experiments conducted within my STSM enabled better understanding of responses to abiotic stresses, provided insight into the mechanisms underlying these responses and the grafting effects using the chlorophyll fluorescence imaging as a tool for assessment of photosynthetic activity in plants under abiotic stress conditions. Besides I learn different physiological parameters related with photosynthesis behavior.

Results

1. Osmotic potential

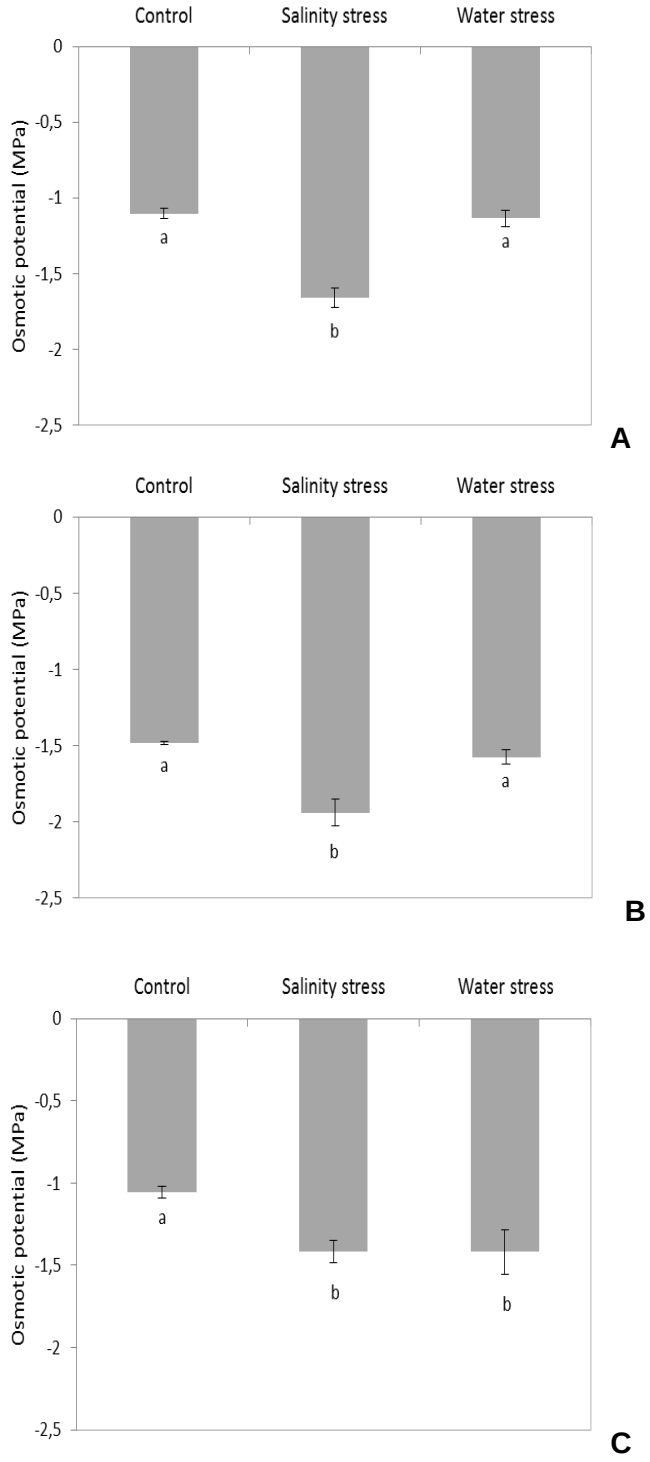
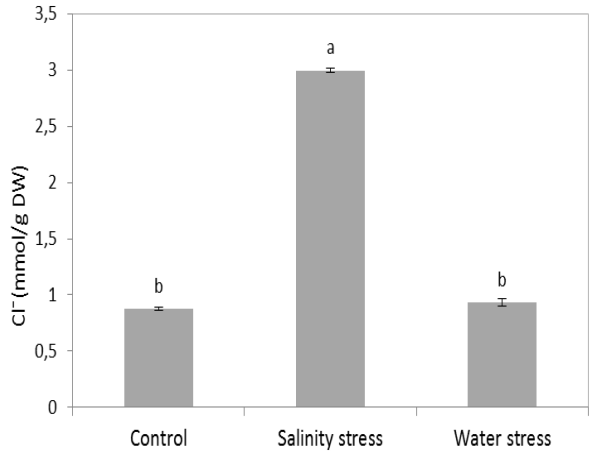
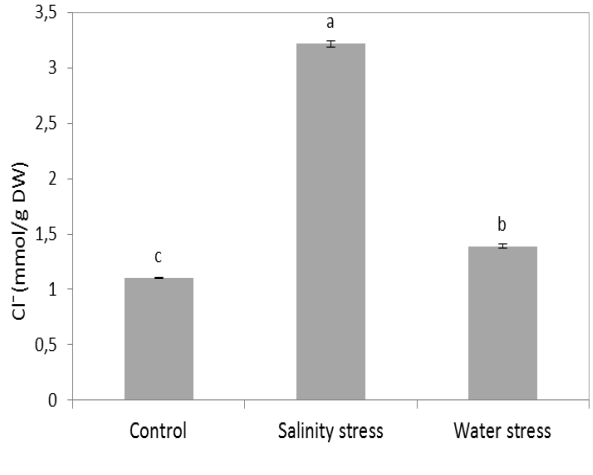


Fig. 7. Leaf osmotic potential (MPa) in eggplant (A), pepper (B) and tomato (C) plants under control conditions, 80 mM NaCl and with 50% water reduction. Dates are mean values for n=4. Different letters indicate significant differences at $P \leq 0.05$ by the LSD test.

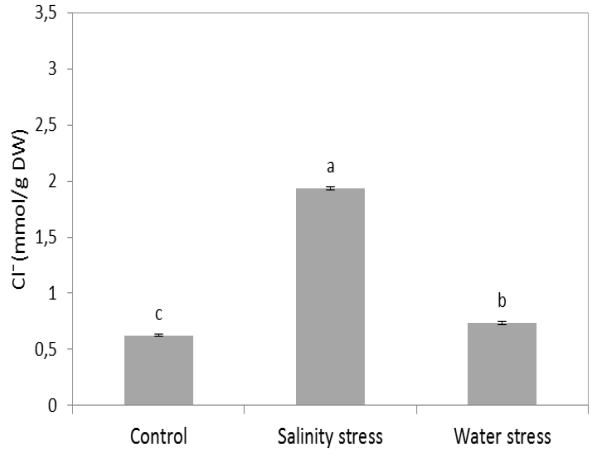
2. The chloride concentration (Cl^-)



A



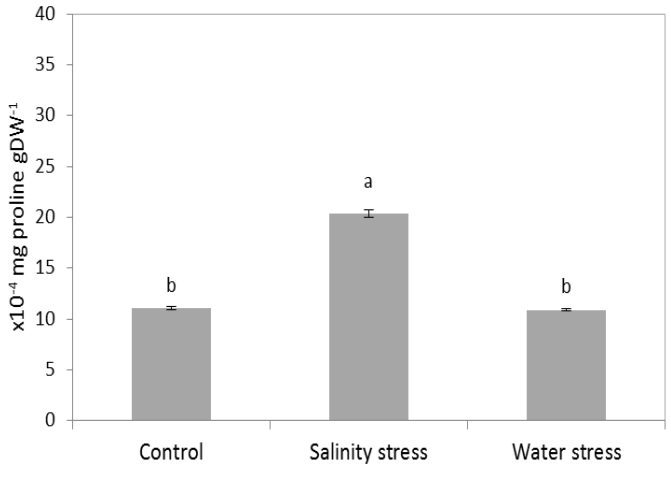
B



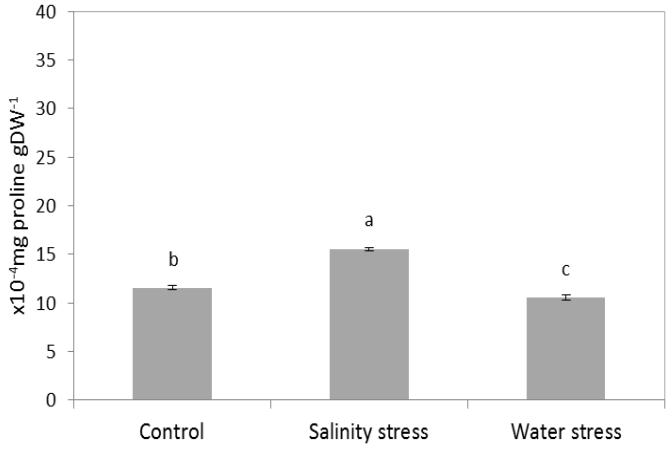
C

Fig. 8. Concentration of Cl^- in mmol/g DW in the leaves of eggplant (A), pepper (B) and tomato (C) plants under control conditions, 80 mM NaCl and with 50% water reduction. Dates are mean values for n=5. Different letters indicate significant differences at $P \leq 0.05$ by the LSD test.

3. Proline content



A



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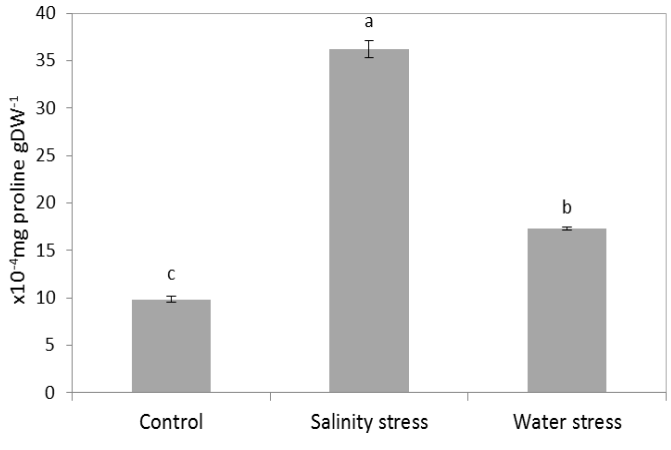
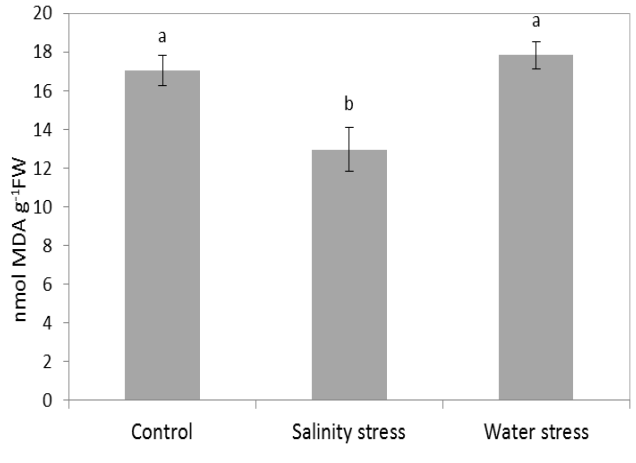


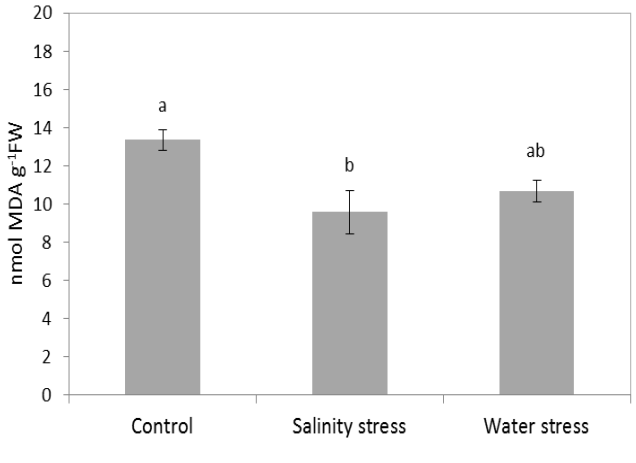
C

Fig. 9. Proline concentration (mg proline/g DW) in the leaves of eggplant (A), pepper (B) and tomato (C) plants under control conditions, 80 mM NaCl and with 50% water reduction. Dates are mean values for n=5. Different letters indicate significant differences at $P \leq 0.05$ by the LSD test.

3. Lipid peroxidation



A



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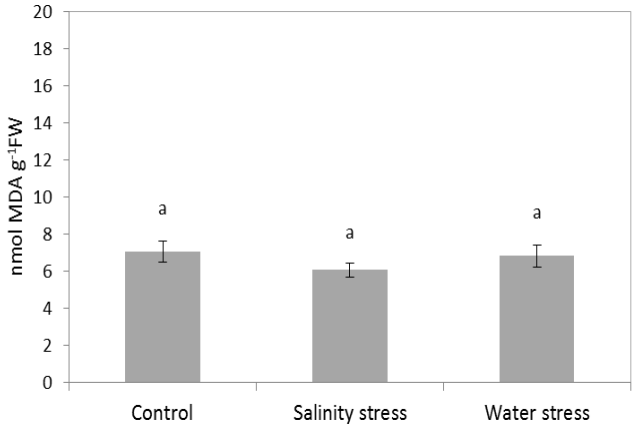

C

Fig. 10. Leaf malondialdehyde content (nmol MDA g^{-1} FW) in the leaves of eggplant (A), pepper (B) and tomato (C) plants under control conditions, 80 mM NaCl and with 50% water reduction. Dates are mean values for n=5. Different letters indicate significant differences at $P \leq 0.05$ by the LSD test.

The equipment used for chlorophyll fluorescence measurements

The WALZ-IMAGING (Walz, Germany) is a highly compact device for measuring images of photosynthetic activity of leaves and other photosynthetic active organisms. Saturation pulse quenching analysis can be applied in a very similar way as with standard PAM Fluorometers. Chl a imaging is a particularly useful tool for detection of heterogeneities due to biotic or abiotic stress. The imaging PAM chlorophyll fluorometer applies pulse – amplitude modulated measuring light to assess Chl fluorescence yield.

Fig. 1. The IMAGING-PAM Chlorophyll Fluorometer (Walz, Germany). The unit represents CCD camera, the multicolour LED light ring array with the leaf under the camera.

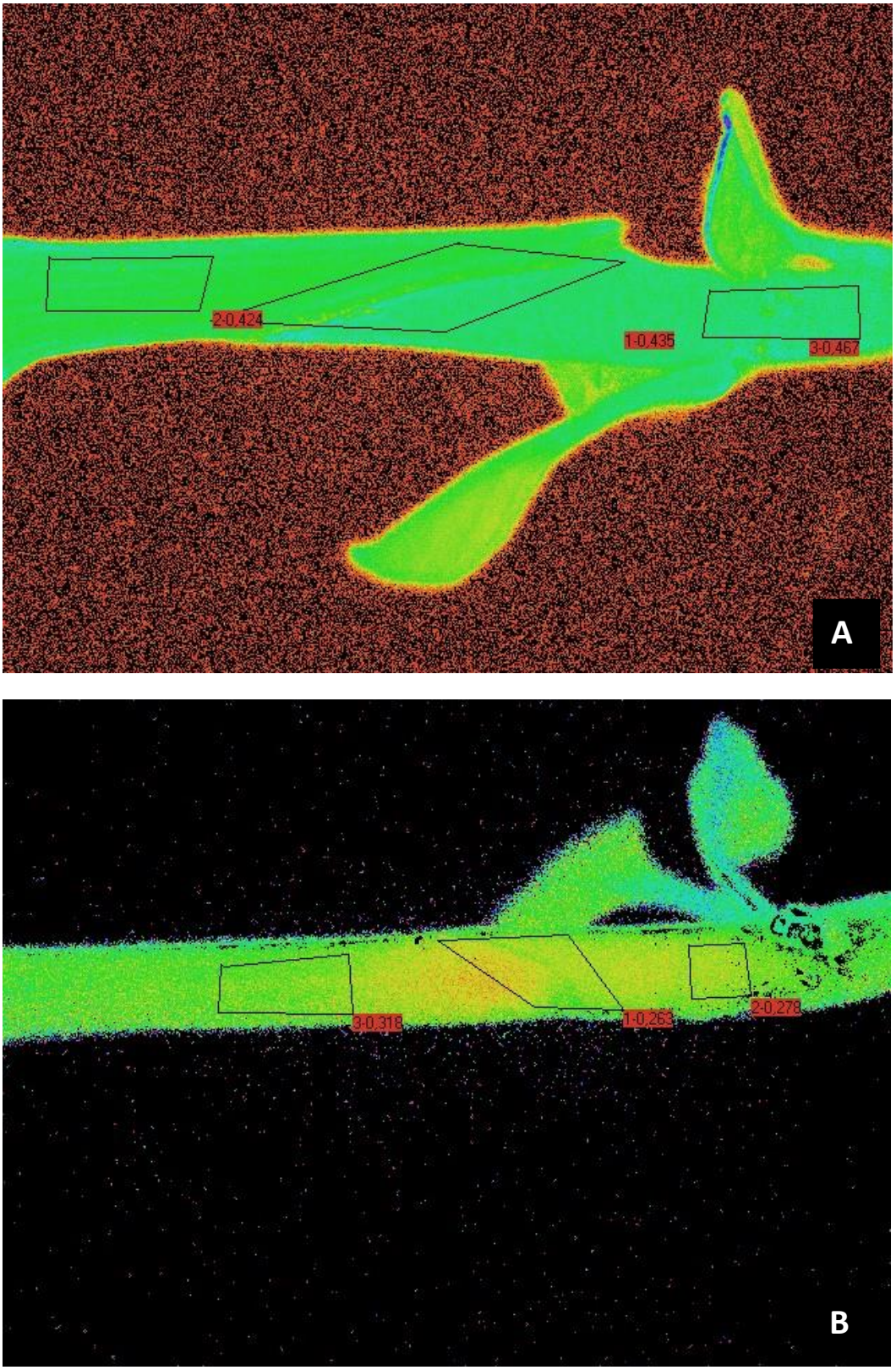

Fig. 2. Fm (A) and Fo (B) images from incompatible graft combination (pepper/eggplant) measured in the graft area (central box), rootstock area (right box) and scion area (left box).

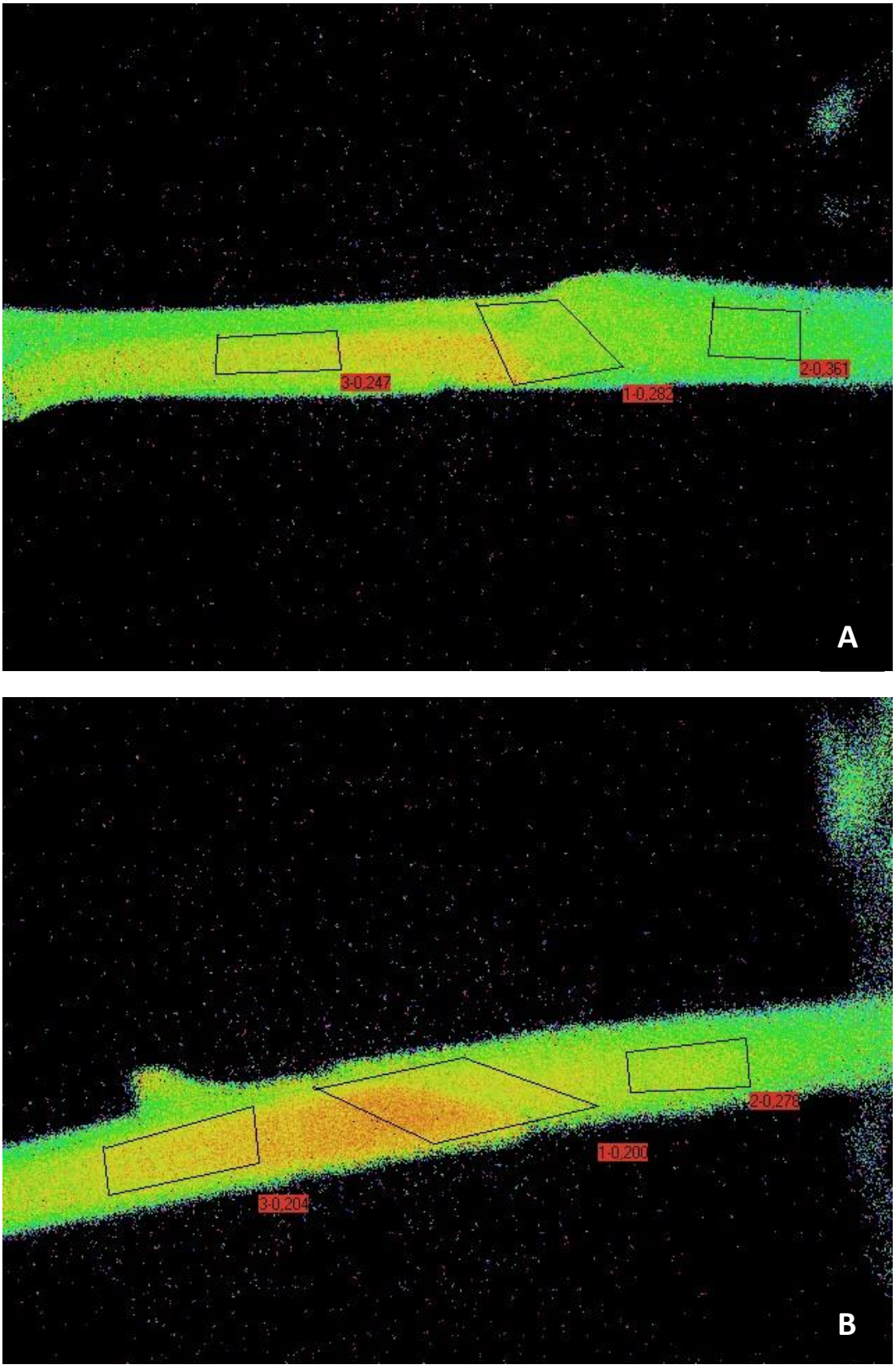

Fig. 3. Fm (A) and Fo (B) images from incompatible graft combination (pepper/tomato) measured in the graft area (central box), rootstock area (right box) and scion area (left box).

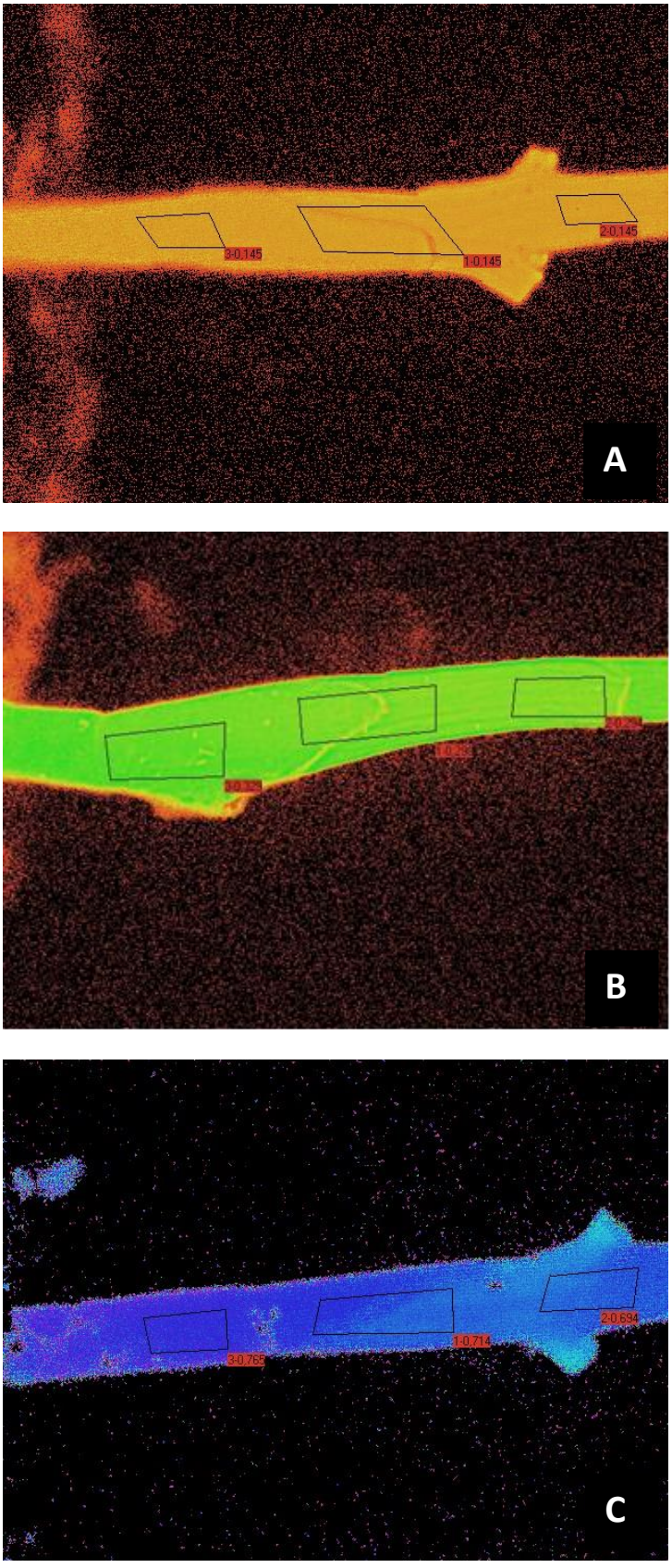

Fig. 4. Fo (A), Fv/Fm (B) and Fm (C) images from compatible graft combination (pepper/pepper) measured in the graft area (central box), rootstock area (right box) and scion area (left box). The Fv/Fm values in the graft area showed higher values compared with the rest of plant combinations.

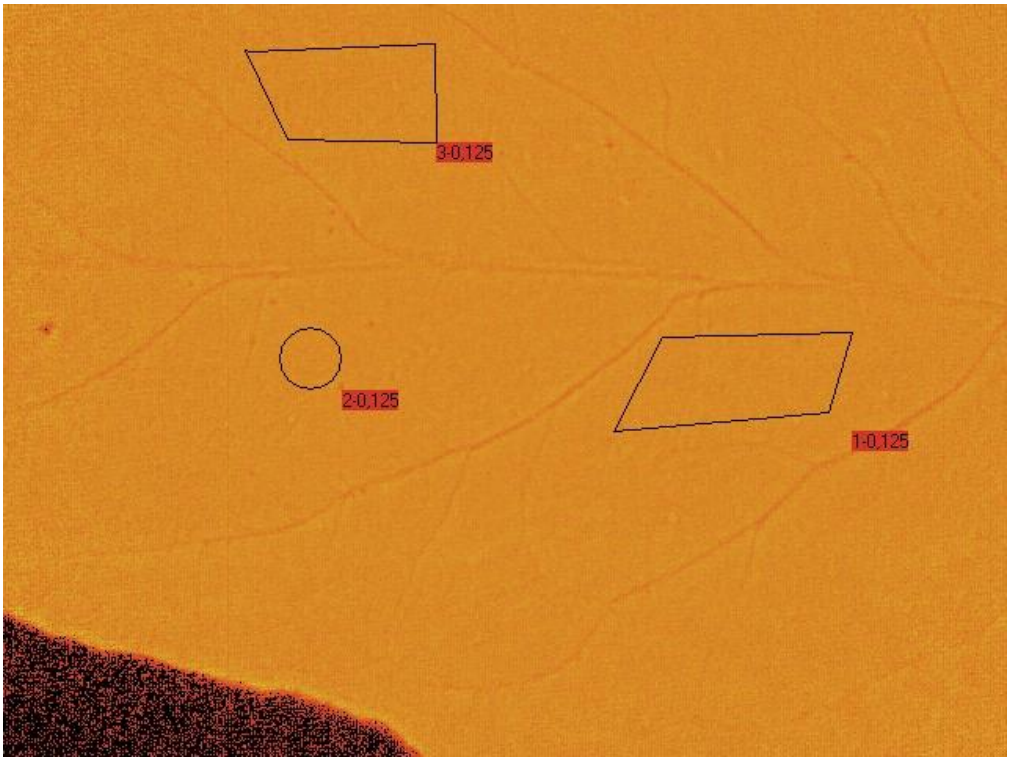

Fig. 5. Fo image from tomato leaf under control conditions.

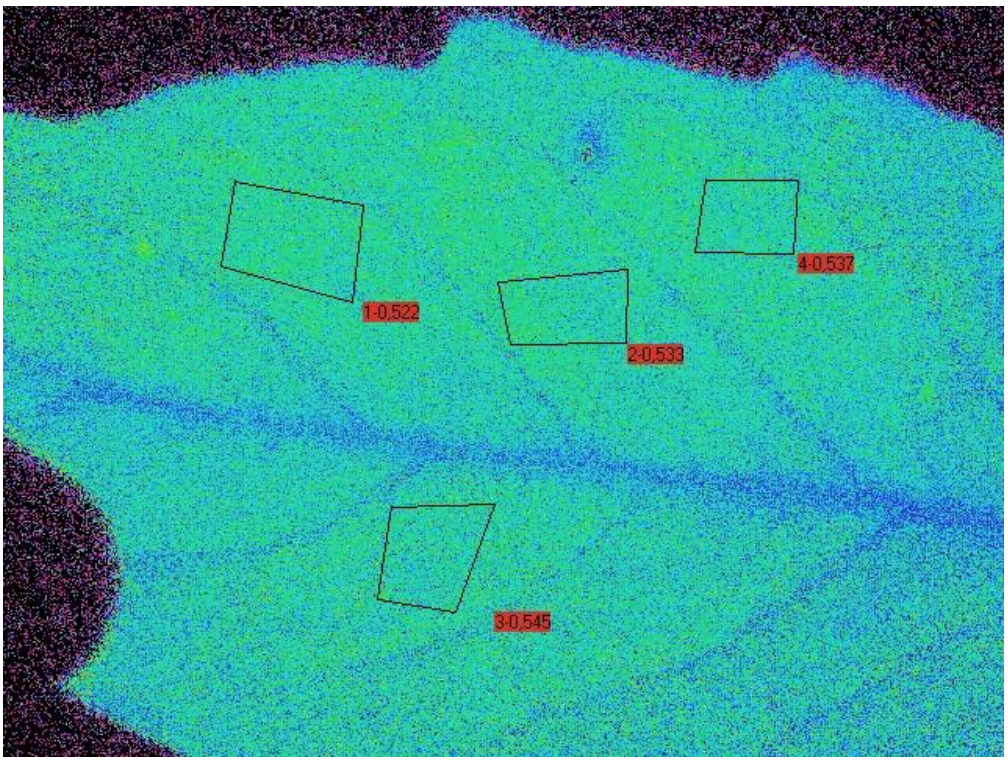

Fig. 6. Fv/Fm image from tomato leaf under salt stress conditions.