COST Action: **FA1204**

STSM title: **Chlorophyll fluorescence imaging (CFI) technique**

Reference: **COST-STSM-ECOST-STSM-FA1204-050514-043925**

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STSM Scientific Report

After completion of the STSM the grantee is required to submit to the host institution and MC Chair (or the STSM coordinator) a short scientific report on the visit **within 4 weeks after his/her stay**.

It should contain the following information:

• purpose of the STSM;

• description of the work carried out during the STSM;

• description of the main results obtained;

• future collaboration with host institution (if applicable);

• foreseen publications/articles resulting or to result from the STSM (if applicable);

• confirmation by the host institution of the successful execution of the STSM;

• other comments (if any).

• **Purpose of the STSM**

The objectives of this FA1204 STSM are:

1) Learn the parameters of Chlorophyll fluorescence imaging (CFI) and its relation to the parameters of photosynthesis. Interpretation of the CFI parameters in grafted pepper plants under water and salinity stress conditions compared with control plants (non-grafted plants).
2) Measurement of fluorescence induction kinetics in grafted and non-grafted plants under different stress conditions.
3) Utilization of CFI technique for monitoring the grafting compatibility/incompatibility in pepper plants in the early stages.
4) Analysis of CFI parameters and selection of appropriate fluorescence parameter that will serve a bioindicator for compatibility in grafted pants.

• **Description of the work carried out during the STSM**

Plant Material

1.1 Salt and water stress effects on pepper grafted plants

The pepper cultivar "Adige" (*Capsicum annuum* var. Lamuyo) was grafted onto different pepper rootstocks – "Adige" (selfgrafted, labeled A/A), hybrid 2 (2/A) and hybrid 6 (6/A). The grafted plants were subjected to drought (35% reduction of irrigation) or salinity stress (80 mM NaCl.) for 7 days. The ungrafted plant cultivar "Adige" was used as control.

Fully expanded mature leaves of plants subjected to drought or salinity stress were measured with Imaging-PAM Chlorophyll Fluorometer (Walz, Germany). The measuring actinic light used was 204 μmol m^{-2} s^{-1} PPFD. The measurements were made in four replicates for every plant type – ungrafted "Adige", A/A, 2/A and 6/A plants.

1.2. Studies of compatibility/incompatibility in pepper grafted plants

The experiment for graft compatibility/incompatibility was performed with the same three types of grafted plants – A/A, 2/A and 6/A. The ungrafted cultivar "Adige" (A) and A/A were used as positive control and "Adige" grafted onto tomato plant (T/A) was used as negative control.

The grafting compatibility/incompatibility in pepper plants in the early stages (10 days after grafted) was monitored with Imaging-PAM Chlorophyll Fluorometer. The areas measured were on the stems in the zone of junction of the rootstock and scion. The actinic light used was 204 μmol m^{-2} s^{-1} PPFD. Eight plants from the ungrafted "Adige", A/A, 2/A, 6/A and the incompatible graft T/A were measured.

1.3. Chlorophyll fluorescence parameters measured.

Chlorophyll fluorescence imaging of pepper leaves or graft area were performed using an Imaging-PAM fluorometer (Walz, Effeltrich, Germany), in order to investigate the spatial-temporal heterogeneity of Chl fluorescence parameters, under water and salt stress conditions. All lights were placed in a ring arrangement and directed at a fixed angle and distance onto the leaf area. Two outer LED-rings (a total of 96 LEDs) provided the measuring light, actinic light and saturating pulse with a peak wavelength at 470nm.

The inner LED-ring (total of 16 LEDs) provided the pulse-modulated light for assessment of PAR-absorptivity at 650nm and a 780nm LED. The charge-coupled device (CCD) camera has a resolution of 640 x 480 pixel. Pixel value images of the fluorescence parameters were displayed with help of a false colour code ranging from black (0.000) through red, yellow, green, blue to pink (ending at 1.000). All measurements were carried out with maximal distance between camera and leaf (26 x 34 mm area). Samples were darkened for 5 min prior to measurement. The minimum (dark) fluorescence Fo was obtained by applying measuring light pulses at low frequency (1 Hz). The maximum fluorescence F_M was determined by applying a saturating blue pulse (10 Hz). The maximum quantum yield of PSII photochemistry Fv/F_M ratio, was determinate as F_M-Fo/F_M. Then, actinic illumination (204 μmol photons m^{-2} s^{-1}) was switched on and saturating pulses were applied at 20 s intervals for 5 min in order to determine the maximum fluorescence yield during the actinic illumination ($F`_M$), and the chlorophyll fluorescence yield during the actinic illumination (Fs). For each interval, saturation pulse images and values of various Chl fluorescence parameters were captured. The values of Fs were normalized to Fo (Fs/Fo) in order to detect water stress early. The quantum efficiency of PSII photochemistry, φ_2, was calculated according to Genty by the formula: $(F`_M\text{-}Fs)/F`_M)$, the φ_2 can be interpreted as the effective quantum yield in PSII photochemistry (φ_p), related to the actual fraction of photochemically active PSII reaction centres (qP) according to Rohácek. The coefficient of photochemical quenching, qP, is a measurement of the fraction of open centres, and is based on a pure puddle antenna model, calculated as $(F`_M\text{-}Fs)/(F`_M\text{-}F`o)$. To determine F`o correctly, it would be necessary to switch off the actinic light and quickly reoxidize the PSII acceptor side with the help of far-red light, but this is not feasible with imaging-PAM as far-red light would penetrate the CCD-detector and cause serious disturbances to fluorescence images (see www.walz.com). The value of F`o was estimated using the approximation of Oxborough and Baker, $F`o = Fo/(Fv/F_M + Fo/F`_M)$. The coefficient of photochemical quenching, qL, is a measurement of the fraction of open PSII reaction centres based on the lake model of PSII antenna pigment organization and it was defined by Kramer as $(F`_M - Fs)/(F`_M - F`o) \times F`o/Fs = qP \times F`o /Fs$.

Calculation of quenching due to non-photochemical dissipation of absorbed light energy (NPQ) was determined at each saturating pulse, according to the equation NPQ= $(F_M - F`_M)/ F`M$ [6]. The quantum yield of regulated energy dissipation in PSII, φNPQ, can be calculated according to Kramer by the equation: φNPQ= 1- φ_2 - 1/(NPQ+1+qL(FM/Fo-1)). For the validity of this equation it is essential that the PSII pigments of the tested samples are organized according to the lake model, which may be assumed for most higher plant leaves. The quantum yield of nonregulated energy dissipated in PSII, φNO, can be calculated according Kramer by the equation: φNO = $1/(NPQ+1+qL(F_M/Fo-1))$. In order to measure homogeneity of excitation light during saturating pulse, actinic light was switch on and the light distribution was measured. A good homogeneity of intensity of excited light was obtained for the whole leaf area.

• **Description of the main results obtained**

<u>Salt and water stress effects on pepper grafted plants</u>

The maximum quantum efficiency of PSII photochemistry was estimated by the ratio Fv/Fm. Fv/Fm slightly decreased upon drought and salinity stress (2-5%) in all plants (A, A/A, 2/A and 6/A, Fig.1). In grafted plants the ratio remained almost unchanged (Fig. 1). The values were higher in controls and lower in drought stressed plants compared with salt treatment.

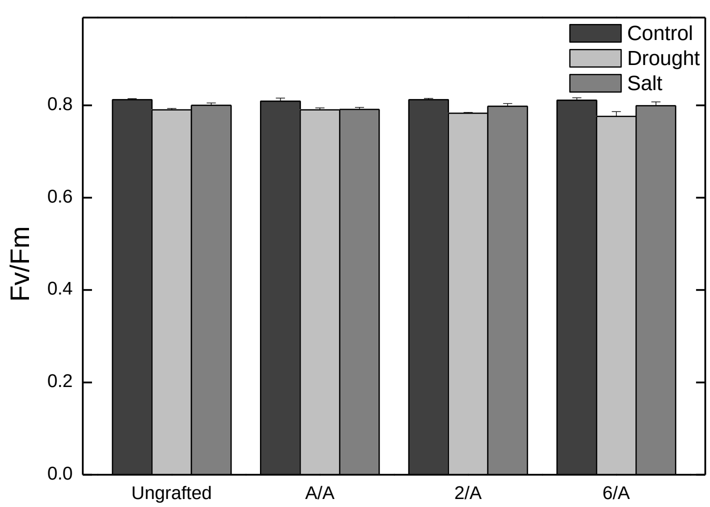

Fig.1. Changes in maximum quantum efficiency of PSII (Fv/Fm) of grafted pepper plants after 7 days under water and salt stress. The values are mean for n=4 samples ±SD

Salinity stress reduced the actual quantum yield of PSII associated with electron transport rate between PSII and PSI in the light-adapted state (ΦPSII) in ungrafted plants (Fig. 2). Grafting didn't change significantly ΦPSII values in all A/A, 2/A and 6/A cultivars. In 2/A and 6/A ΦPSII was affected by drought and salinity stress and slightly decreased.

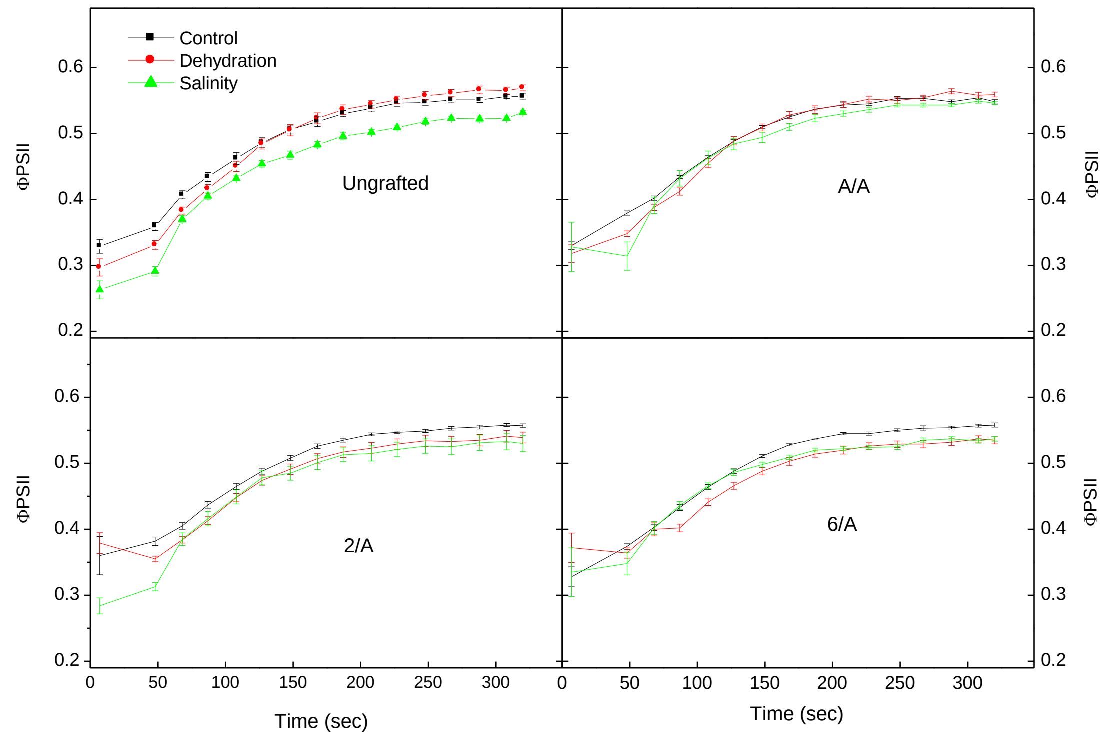

Fig. 2. Induction kinetic curve of the actual quantum yield of PSII associated with electron transport rate between PSII and PSI in the light-adapted state (ΦPSII) of grafted pepper plants after 7 days under water and salt stress. The values are mean for n=4 samples ±SD

The thermal energy dissipation, expressed as non-photochemical quenching (NPQ), increased in all plant in the first 50 sec mainly in the plants subjected to drought and salt stress, compared to controls with a fast quenching (Fig. 3). At the end of induction kinetics, the values of NPQ in ungrafted plants were higher under salinity stress indicating that the energy goes to thermal dissipation and in minor degree to photosynthesis. In grafted plants the values at the end of kinetics were similar to lower to control. These results showed that grafted plants have a higher capacity to drive the energy to photosynthesis.

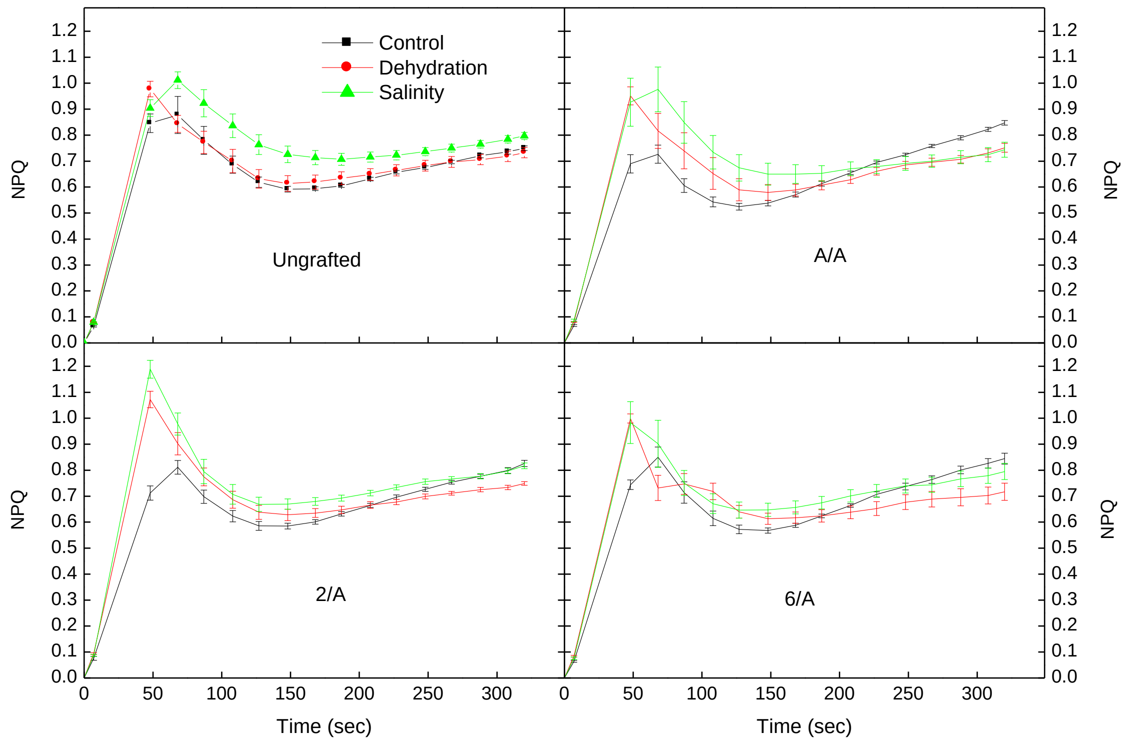

Fig. 3. Induction kinetic curve of the thermal energy dissipation, expressed as non-photochemical quenching (NPQ) of grafted pepper plants after 7 days under water and salt stress. The values are mean for n=4 samples ±SD

The fraction of open reaction centers, based on lake model, qL, was higher in all drought stressed plants, especially in ungrafted (Fig. 4). In grafted controls and salinity stressed A/A, 2/A and 6/A plant combinations, qL increased compared to ungrafted plants, while in drought stressed grafts the values were lower than in ungrafted ones.

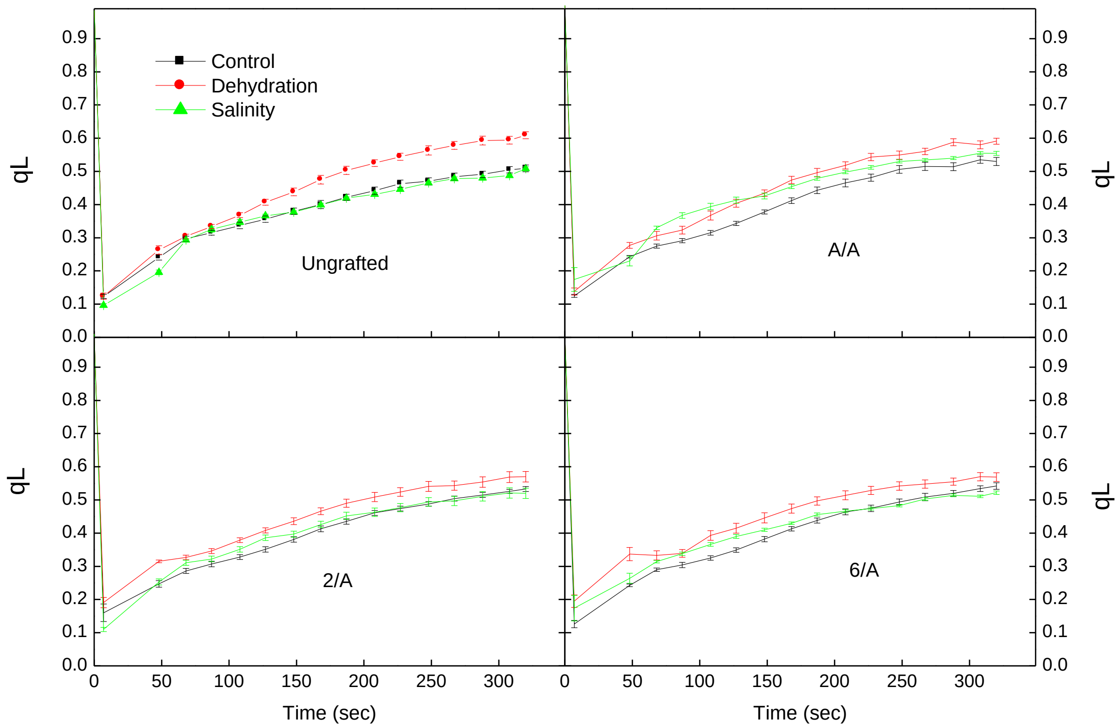

Fig. 4. Induction kinetic curve of the fraction of open reaction centers, based on lake model, qL, of grafted pepper plants after 7 days under water and salt stress. The values are mean for n=4 samples ±SD

Studies of compatibility and incompatibility in pepper grafted plants

The measurements of chlorophyll fluorescence for grafting compatibility/incompatibility showed that grafted A/A, 2/A and 6/A plants possessed a higher values for Fv/Fm, ΦPSII and parameters of photochemical quenching and lower for the non-photochemical processes in area of scion compared to the zone of junction and rootstock, suggesting a good compatibility between the rootstocks and the scion “Adige”. The negative control T/A grafts showed the opposite results.