IECHo 2025 Conference

The 2nd International Electronic Conference on Horticulturae

27–29 May 2025 | Online

MDPI

Photosynthetic Damage In Citrus Leaves Under Freezing Stress Revealed By OJIP Chlorophyll Fluorescence Kinetics

Carlos Eduardo Aucique-Perez[1], Shahid Iqbal[1], Muhammad A. Shahid[1]*

[1]UF/IFAS, Horticultural Sciences Department, North Florida Research and Education Center, Quincy, FL 32351, USA

INTRODUCTION & AIM

Cold stress poses a significant threat to citrus production and the establishment of new orchards in northern Florida. Low temperatures reduce the photosynthetic capacity of citrus plants due to both stomatal and non-stomatal limitations, often resulting from structural damage to cell membranes. Studies have demonstrated that freezing stress in plants primarily causes chloroplast damage, photoinhibition, inactivation of Calvin cycle enzymes, oxidative stress, and functional disturbances in vascular tissues. Chlorophyll *a* fluorescence (CF) is a non-invasive and non-destructive method that has been successfully used to study the photosynthetic responses of plants under various environmental conditions. Therefore, changes in CF characteristics can serve as reliable indicators of various stresses, including nutrient deficiencies, abiotic and biotic stresses. The OJIP induction curve, commonly known as the OJIP-test, capture the complex, nonlinear dynamics of photosystem II (PSII) in response to light excitation, including energy absorption, transfer, dissipation, and photochemical reactions. Based on this perspective, the present study was conducted to gain insight into how the photosynthetic machinery of citrus leaves is affected by freezing temperatures, using the method of OJIP fluorescence transients.

MATERIALS AND METHODS

Citrus plants were sprayed with two biostimulants [Proline (Pro; 50 mM)and Methyl-jasmonate (JA; 50 mM)] separately and in combination, with four applications over a two-week period. Following these treatments, the plants were transferred to field conditions two days before natural freezing event (Fig. 1). Control plants, maintained at 25°C, received the same biostimulant treatments. After freezing stress, three leaves per plant per treatment were collected and kept in the dark for 60 minutes. The OJIP-test was then performed to measure fluorescence kinetics using a Li-Cor 6800 infrared gas exchange system equipped with a fluorescence chamber (Fig. 2).

Stress induction

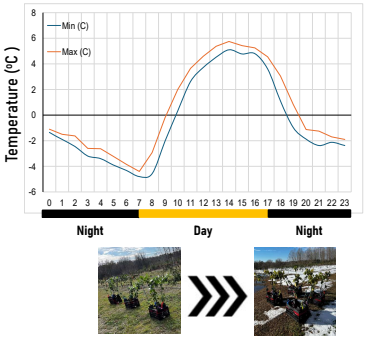

Figure 1. Maximum and minimum temperatures in the north of Florida during the winter season in 2025.

Determinations

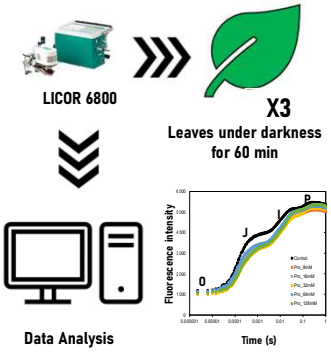

Figure 2. Fluorescence kinetics (OJIP-test) protocol used in leaves of citrus treated with biostimulants and exposed to freezing conditions in 2025.

RESULTS & DISCUSSION

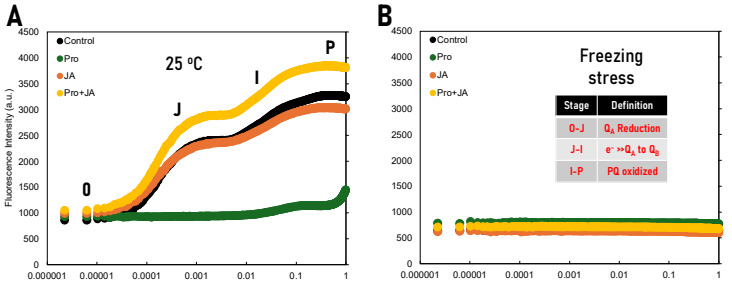

Figure 3. Fluorescence intensity detected in citrus leaves treated with two biostimulants (Pro and JA) separately and in combination under normal temperature (25ºC) and freezing stress in field conditions using the OJIP test. n = 4

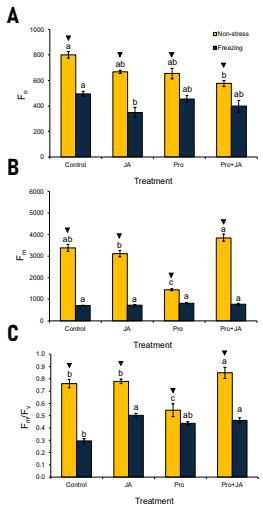

Figure 4. Fluorescence parameters (F_o, F_m, and F_v/F_m) derived from the OJIP test in citrus leaves treated with two biostimulants (Pro and JA), applied separately and in combination, under normal (25°C) and freezing field conditions. Data represent the mean of four replicates (n = 4).

From the results obtained, the OJIP test detected significant alterations in the fluorescence intensity pattern in both biostimulants-sprayed leaves and stress conditions. At 25 °C, plants sprayed with JA and water showed a similar fluorescence intensity pattern, while combination (Pro+JA) showed a fluorescence pattern higher than other treatments. Curiously, Pro caused reductions in the fluorescence intensity in citrus leaves (Fig. 3A). On the other hand, freezing conditions suffered by plants for 3 days caused a minimal fluorescence emission, suggesting damage to the photosynthetic machinery (Fig. 3B).

F_o, F_m, and F_v/F_m values were significantly reduced in all treatments exposed to freezing conditions (Fig 4A to C). F_o and F_v/F_m values showed significant differences among treatments under freezing stress (Fig 4 A and C). At 25 °C, leaves sprayed with Pro showed a significant reduction in all fluorescence parameters (Fig. 4).

CONCLUSION

1. The OJIP test provides a fast, reliable, and sensitive measure of photosynthetic capacity in citrus leaves under freezing stress.
2. This methodology successfully identified fluorescence intensity changes in leaves treated with biostimulants, reflecting potential impacts on their photosynthetic function.
3. Parameters derived from the OJIP-test highlighted the effects of both freezing stress and biostimulants application on citrus leaves.

FUTURE WORK

The OJIP test holds significant promise for future research on freeze tolerance in citrus, offering a powerful, non-invasive tool to detect early physiological changes and evaluate the effectiveness of stress-mitigation strategies such as biostimulant applications.

CONTACT US

Fruit Physiology Lab NFREC UF IFAS

* Dr. Muhammad Adnan Shahid
Assistant Professor
mshahid@ufl.edu

Dr. Carlos E. Aucique-Perez
Postdoctoral Associate
c.aucique perez@ufl.edu

UF IFAS UNIVERSITY of FLORIDA