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Monitoring of plants sensitivity to physiological active compounds and stress factors with fluorescent methods

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Received December 01, 2023
Revised January 17, 2024
Accepted March 05, 2024

Induced changes in the fluorescence of potato and marigold leaves were studied after treating the tubers (potatoes) and spraying the vegetating plants (marigolds) with the growth regulator „Epin-Extra“ and the silicon-containing liquid organic fertilizer „Siliplant“. The use of these compounds made it possible to compensate for the negative impacts on the photosynthetic apparatus of the plants, associated with the treatment of potato tubers with the fungicide „Maxim“as well as the exposure of marigold plants to a temperature of 5°C for three days.

Keywords: potato plant, marigolds, fungicides, epin, siliplant, chlorophyll fluorescence.

DOI: 10.61011/EOS.2024.04.58875.27-24

Introduction

Fluorescent indices of photosynthetic objects depend on a wide range of biotic and abiotic factors and are particularly appealing as means for assessing the structural and functional organization of the photosynthetic apparatus of plants [1–4]. Of outstanding interest are the inductive changes in fluorescence that are observed after a short period of darkness and are reflective of regulatory changes in photosynthesis. It has been demonstrated earlier that relative changes in the $(F_M - F_T)/F_T$ index of slow fluorescence induction (SFI; the degree of fluorescence quenching within the induction period) correspond to relative changes in the photosynthetic activity of plants assessed by the rate of gas exchange per chlorophyll [5,6]. The SFI method was applied successfully in studies into the functional activity of the photosynthetic apparatus of plants under various physiological conditions [7–9]. It appears important and relevant to obtain additional information about the information capabilities of fluorescent indices of plants under various physiological conditions.

In the present study, the fluorescent indices of potato and marigold plants were investigated after the treatment of tubers (potatoes) and vegetative plants (marigolds) with the „Epin-Extra“ and „Siliplant“ agents. „Epin-Extra“ (its active ingredient is epibrassinolide, a steroid phytohormone) is used widely in plant cultivation to facilitate the growth and development of plants, raise their resistance to adverse environmental factors (including cold stress), and shape economically valuable traits. „Siliplant“ is a silicon-containing liquid organic fertilizer that increases the efficiency of herbicides and reduces their phytotoxicity. The stimulating effect of these agents on the photosynthetic activity and crop yields has been demonstrated earlier in [10,11].

The problems discussed in the present study are practically relevant.

(1) It is a common practice in seed potato growing to treat tubers with fungicides during storage and before planting [12]. At the same time, it is known that such treatment may have a negative effect on biochemical parameters and cause a reduction in productivity of potatoes of the next generation [13]. In this regard, we intended to find out whether the treatment of tubers with protective and stimulating agents of biological nature can compensate for the negative aftereffect of fungicides on the photosynthetic apparatus of plants grown from these tubers.

(2) Decorative marigold plants are used widely in urban landscape design. They are fairly resistant to adverse conditions and are characterized by active flowering from early summer to late autumn. The aim of the present study was to find out whether treating marigolds with physiologically active substances could increase their resistance to low temperatures and, consequently, extend their flowering period.

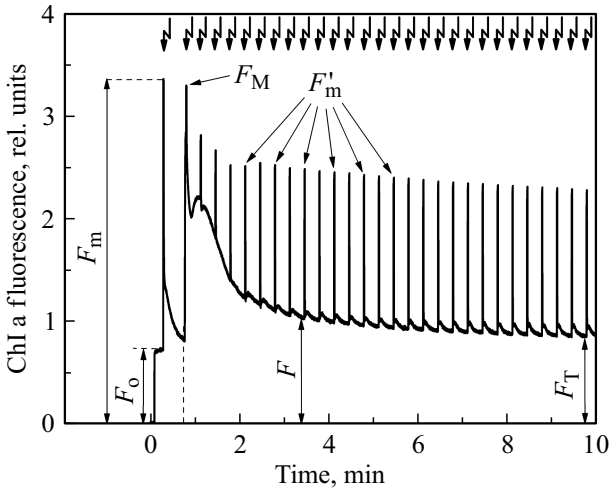
These aims were achieved by examining the fluorescent indices of plants reflecting the functional activity of the photosynthetic apparatus. A single-beam arrangement was used to record the fluorescence of potato leaves. In these experiments, sufficiently intense fluorescence-exciting light initiated the corresponding inductive changes in the primary processes of photosynthesis that depended on the oxidation-reduction state of carriers of electrons between two photosystems (PSs), the proton gradient on the thylakoid membrane, and the nature of the excitation energy distribution between PSs [1,2]. One of the mechanisms

affecting the intensity of chlorophyll fluorescence is the movement of chloroplasts within the cell (the so-called „scattering“ of chloroplasts under the influence of sufficiently intense light) [14]. In the case of marigolds, a pulse fluorometer was used. The chlorophyll fluorescence excited by weak measuring light in these experiments reflected the changes induced by intense acting (actinic) light. A similar measurement arrangement supplemented with high-power saturating light pulses allows one to estimate the quantum efficiency of photochemical transformations in PS2 and determine the coefficients of photo- and non-photochemical fluorescence quenching in the induction period [15].

1. Objects and methods

Potato plants *Solanum tuberosum* and marigolds *Tagetes patula L* were studied. Prior to storage, potato tubers were treated with the „Maxim“ fungicide and the „Epin-Extra“ and „Siliplant“ agents in the recommended doses. At the end of the storage period, tubers were planted in pot and field experiments. In order to record fluorescence, plant leaves were separated from the stem, secured in a special holder, and kept in the dark for 5 minutes to standardize the experimental conditions. A leaf was then illuminated with broadband blue light with an approximate intensity of 100 W/m^2; fluorescence was recorded at a wavelength of 686 nm. The $(F_M - F_T)/F_T$ ratio, where F_M is the maximum fluorescence intensity achieved after approximately 20 s of illumination and F_T is the steady fluorescence level reached after 10–15 min of illumination, was used as the SFI parameter.

Marigold plants were sprayed with aqueous solutions of „Epin-Extra“ (concentration: 0.2 ml/l) and „Siliplant“ (3 ml/l) in the active flowering phase; control plants were sprayed with water. On the next day after treatment, containers with plants were introduced into a refrigerator and kept there at a temperature of 5°C for 3 days. Another batch of water-treated plants was kept under substantial shading at room temperature. The kinetics of chlorophyll fluorescence in marigold leaves was measured with a PAM-2500 (Walz, Germany) pulse fluorometer. A cut portion of a leaf was secured in a holder and kept in the dark for 5 min. The protocol for fluorescence measurements is presented in the figure. Fluorescence was excited by pulsed measuring light ($\lambda = 630$ nm, $\Delta\lambda = 5$ nm, and $I = 10\,\mu\text{E}\,\text{m}^{-2}\text{s}^{-1}$); initial fluorescence level F_0 was determined immediately after turning on the measuring light. Maximum level F_m was determined by illuminating the leaf with a saturating flash of light ($\lambda = 630$ nm, $\tau = 0.5$ ms, and $I = 3400\,\mu\text{E}\,\text{m}^{-2}\text{s}^{-1}$). Induction changes in fluorescence were observed when the acting light ($\lambda = 455$ nm and $I = 150\,\mu\text{E}\,\text{m}^{-2}\text{s}^{-1}$) was turned on; saturating flashes of light followed at intervals of 20 s. The following parameters were measured: $\Phi_{PSII} = (F'_m - F)/F'_m$ (characterizes the effective quantum yield of PS2) and $\Phi_{NPQ} = (F_m - F'_m)/F'_m$ (non-photochemical fluorescence quenching coefficient).



Protocol for fluorescence measurements with a PAM fluorometer. Zigzag arrows indicate the moments when saturating flashes of light are switched on.

These values were determined with fluorescence already at its steady level F_T. The $(F_M - F_T)/F_T$ ratio was also calculated.

2. Results and discussion

2.1. Experiments with potatoes

In the pot experiment, SFI parameter $(F_M - F_T)/F_T$ of leaves of plants grown from tubers treated with the fungicide decreased compared to the control level, whereas the parameter values determined in all experiments involving the use of immunomodulators increased. The most profound enhancement corresponded to a mixture of „Maxim“ and „Epin-Extra“ (see Table 1).

These data are indicative of an increase in the functional activity of the photosynthetic apparatus of plants under the influence of immunomodulators. It is significant that the differences in photosynthetic activity revealed by the SFI method in experiments with various types of tuber treatment corresponded to the differences in yield of potatoes of the next generation (Table 1). Specifically, the lowest values of the $(F_M - F_T)/F_T$ SFI index and the lowest yield were observed when tubers were treated with the „Maxim“ fungicide, and the highest values of $(F_M - F_T)/F_T$ and the highest yield were obtained after treatment with a mixture of fungicide and epin. A strong positive correlation between the $(F_M - F_T)/F_T$ SFI values of plants recorded in the pot experiment and the potato yield in the field experiment was established. The correlation coefficient for $n = 6$ pairs of values listed in the table was $r = 0.95$; the correlation is significant with probability $p > 0.999$.

Table 1. Fluorescent indices of potato leaves after the treatment of tubers with the „Maxim“ fungicide and the „Epin-Extra“ and „Siliplant“ agents and yield of potatoes of the next generation (standard deviations are indicated in the column for $(F_M - F_T)/F_T$)

Types of tuber treatment	$(F_M - F_T)/F_T$ (pot experiment)	Yield, t/ha, HCP_{05} 0.2 (field experiment)
Control	0.38 ± 0.03	32.5
„Maxim“	0.31 ± 0.02	29.0
„Epin-Extra“	0.44 ± 0.02	32.4
„Siliplant“	0.43 ± 0.02	33.4
1/2„Maxim“+„Epin-Extra“	0.58 ± 0.03	41.8
1/2„Maxim“+„Siliplant“	0.56 ± 0.03	39.6

Table 2. Fluorescent indices of marigold plants treated with the „Epin-Extra“ and „Siliplant“ agents: Φ_{PSII} (± 0.005), NPQ (± 0.02), and $(F_M - F_T)/F_T$ (± 0.05) (maximum standard deviations are indicated)

Treatment	Before cooling			After cooling		
Type	Φ_{PSII}	NPQ	$(F_M - F_T)/F_T$	Φ_{PSII}	NPQ	$(F_M - F_T)/F_T$
Water	0.530 (100%)	0.60 (100%)	1.75 (100%)	0.465 (88%)	0.54 (90%)	1.50 (86%)
„Epin-Extra“	0.520 (100%)	0.59 (100%)	2.10 (100%)	0.515 (99%)	0.63 (107%)	2.05 (98%)
„Siliplant“	0.520 (100%)	0.54 (100%)	1.80 (100%)	0.520 (100%)	0.59 (109%)	1.85 (103%)

2.2. Experiments with marigolds

All the measured fluorescent indices decreased significantly in the group of control marigold plants kept in the dark at low temperature (Table 2). At the same time, no significant changes in these indices were observed for plants that were kept in the dark at room temperature (data not shown). Thus, cold stress was a significant factor affecting fluorescence indices. After cooling, significant (95% CL) differences in all the examined indices between the treated and control groups were revealed by one-way analysis of variance (Table 2).

According to literature data, photosynthesis is one of the physiological processes most sensitive to low temperatures [16]. It is also known that PS2, which drives the fluorescence of photosynthetic objects, is the system most sensitive to stresses [17]. Thus, it is fair to assume that the reduced fluorescent indices of marigold leaves from the control group measured after three days of cold stress are indicative of disturbances in the structural and functional organization of the photosynthetic apparatus. Specifically, the efficiency of light energy utilization in PS2 (parameter Φ_{PSII}) decreases, the mechanisms of formation of the proton gradient on thylakoid membranes (parameter NPQ) get disrupted, and, consequently, the photosynthetic activity (parameter $(F_M - F_T)/F_T$) decreases. It is significant that the treatment of plants with the „Epin-Extra“ and „Siliplant“ agents increased the resistance of the photosynthetic apparatus to low temperatures, since no significant changes in fluorescent indices were observed after cooling in these cases (Table 2).

3. Conclusion

The obtained results suggest that fluorescent methods based on the detection of induction changes in chlorophyll fluorescence *in vivo* may be used in the drafting of measures to enhance the environmental safety of chemicals and the resistance of plants to biogenic and abiogenic stresses. These methods compare favorably with longer and more labor-intensive biochemical studies that are carried out to solve applied problems of plant physiology.

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by D.Safin