

Photosynthetic response of some *Triticum cultivars* to the combined influence of nanofertilizers and water deficit

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ABSTRACT

The effects of water deficit modelling by polyethylene glycol (PEG), metal nanoparticles (NPs) and their combined action on water status, chlorophyll fluorescence and pigments composition in the leaves of three drought tolerant wheat genotypes – *Triticum aestivum* L. (Trypilska and Favorytka cultivars) and *Triticum dicoccum* Schrank. (cv. Holikovska), have been studied. Water deficit, induced by PEG, as well as the treatment with metal NPs (Cu, Fe, Mn, Zn) and their combined action, showed no effects on the maximal quantum yield of PSII photochemistry (F_v/F_m) in dark-adapted leaves of studied plants. However, water deficit decreased the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m') in light-adapted leaves of the Favorytka cultivar. The increase of chlorophyll fluorescence photochemical quenching (qP) and actual quantum yield of PSII electron transport (ϕ PSII) levels, the decrease in non-photochemical quenching coefficient (qN) were observed in Favorytka and Holikovska cultivars under the combined action of water stress and NPs treatment. According to photosynthetic pigments composition, the NPs treatment of wheat plants, grown on medium with PEG, did not bear an additional pressure on the seedlings. However, the NPs treatment did not decrease the negative effect of the simulated water deficit of the cv. Favorytka seedlings.

Keywords: *Triticum*, water deficit, metal nanoparticles, photosynthetic response, chlorophyll fluorescence

INTRODUCTION

Plants are frequently subjected to various abiotic stressors due to unfavorable environmental conditions that affect their metabolism, growth, development and crop yield capacity. It has been estimated that more than 50% of yield reduction is the direct result of abiotic stressors (Thiry et al., 2016). Drought is the most widespread environmental factor limiting wheat productivity in many regions of its cultivation. Because of the essential role of water in metabolism, any decrease in water availability leads to biochemical, physiological and morphological responses at the cellular and whole-plant level (Babenko et al., 2019; Smirnov et al., 2020a). Plant photosynthesis is highly susceptible to water

stress. Reduction in net photosynthetic rate is attributed to both stomatal and non-stomatal control (Yang et al., 2009). Plants react to water deficit with a rapid closure of stomata to avoid further water loss via transpiration. Consequently, CO₂ diffusion into leaf is restricted. Non-stomatal responses of photosynthesis under water stress are attributed to inhibition of photosystem II (PSII), which is manifested by the decreasing of chlorophyll a fluorescence parameters, electron transport through PSII, and pigment content (Yordanov et al., 2003). The ability to maintain the functionality of the photosynthetic apparatus (PSA) under water stress is of major importance for plant water deficit tolerance.

The most current practice in sustainable environmental management of agriculture is to find ways to diminish the impact of drought stress on crop production (Schröder et al., 2019). One of the conceptual approaches to increasing the adaptive potential of cultivated plants is using new environmentally friendly approaches that do not require large expenditures (Zulfiqar et al., 2019; Marthandan et al., 2020). They should be based on promoting the plant capacity of adaptation to drought (Kang et al., 2009; Taran et al., 2017). Nowadays, colloidal solutions of essential metals are widely used in agrotechnologies to increase plant productivity through enhancing the adaptive capacity to drought. The use of NPs colloidal solutions is interesting for increasing wheat productivity under water stress (Kashyap et al., 2020; Ahmed et al., 2021).

Therefore, this work aimed to investigate the influence of NPs colloidal solution (Cu, Fe, Mn, Zn) on chlorophyll fluorescence and photosynthetic pigments content in common bread wheat cultivars (*Triticum aestivum* L.) and emmer wheat (*Triticum dicoccum* Schrank.), grown under PEG-induced water deficit.

MATERIAL AND METHODS

Plant material and water deficit modelling

To investigate photosynthetic processes under the combined influence of nanofertilizers and water deficit, three wheat genotypes (cv. Trypilska, cv. Favorytka (*Triticum aestivum* L.) and cv. Holikovska (*Triticum dicoccum* Schrank.)) were used. Seedlings were grown on distilled water in a growth chamber at 25 °C with photoperiod 16/8 h, at 200 $\mu\text{mol}/\text{m}^2\text{s}$ of photon flux density for 7 days. Soil water stress was simulated by adding polyethylene glycol (PEG) 6000 to the growth medium to adjust osmotic potential up to -0.3 MPa. In the variants with an application of NPs, seeds were pre-soaked in the experimental solution of NPs for 24 h. Control plants were treated using distilled water.

Colloidal solutions of NPs were developed by the Department of the Technologies of construction materials and materials science of the National University of Life and Environmental Sciences of Ukraine. NPs were

obtained as a result of dispersing Cu, Fe, Mn and Zn granules by impulses of electric current with amplitude of 100–2000 A (Lopatko et al., 2009). The experimental mixture of essential metals NPs contained CuNPs, FeNPs in a concentration of 4 mg/L and MnNPs, ZnNPs in a concentration of 3 mg/L.

Relative water content

Measurements of relative water content (RWC) in leaves was determined according to Tanentzap et al. (2015) and calculated as: $\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) * 100$, where FW means the fresh weight of leaf segments, TW is a weight at full water saturation measured after their 4 h rehydration in distilled water, and DW is a constant leaf dry weight after 12 h drying at 105°C.

Photosynthetic pigments

The fresh leaf tissues (0.2 g) were homogenized together with 0.5 g of glass sand and 0.5 g of Na_2SO_4 with mortar and pestle and filtered with 96% ethanol through filter funnel. The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) were measured using a UV-vis spectrophotometer (Shimadzu 1800, Japan) at 665, 649 and 440.5 nm respectively. A solution of 96% ethanol was used as a blank. Concentrations of Chl *a*, Chl *b*, and Car were calculated from the equations as described by Lichtenthaler (1987).

Chlorophyll fluorescence

Chlorophyll *a* fluorescence was measured using a XE-PAM fluorometer (Walz, Germany). The leaves were held in the dark for about 20 min before measurement. Minimal fluorescence (F_0) of dark-adapted leaves was determined at low photon flux density (near 0.2 μmol (quantum)/ m^2s). Maximal fluorescence (F_m) of dark-adapted and light-adapted (F'_m) leaves was detected at saturating irradiance (1 s) of halogen lamps (5000 μmol (quantum)/ m^2s). The intensity of the actinic light was 200 μmol (quantum)/ m^2s (as for the cultivation of plants). Fluorescence induction parameters F_v/F_m , F'_v/F'_m , qP , qN , ϕPSII were calculated as described by Murchie and Lawson (2013).

Data analysis

The experiment was conducted in three biological and analytical repeats. For computing the data, an analysis of variance (ANOVA) with Duncan's multiple range test was performed. Data are expressed as means followed by standard deviation and were considered reliable at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Effects of water deficit on seedlings water status

Model experiments were conducted to understand the response of plants to water deficit. One of the indicators of both the sensitivity and the resistance to water deficit in plants is the leaf relative water content (RWC). It was shown that PEG induced the decline of RWC in leaves of all genotypes (Figure 1).

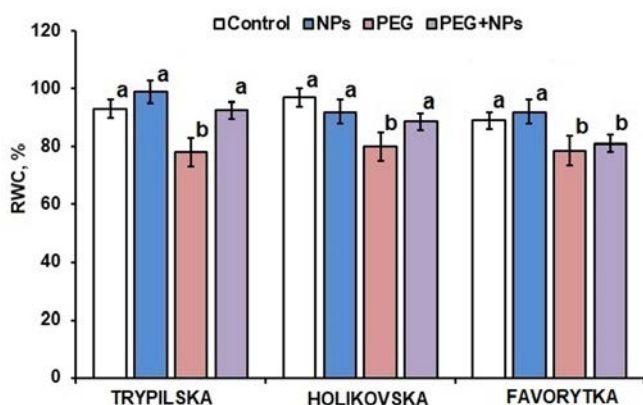


Figure 1. The relative water content (RWC) of wheat leaves under pre-soaked application with metal nanoparticles (NPs), grown with polyethylene glycol (PEG), and their combined action; $n=50$ (means followed by the same letters were not significantly different at $P < 0.05$ according to the Duncan's multiple range test)

RWC decreased from 93%, 97% and 89% in seedlings of control treatment of Trypilska, Holikovska and Favorytka cultivars to 78%, 80% and 82% in water-stressed plants, respectively. NPs treatment of plants resulted in the restoring of water balance in leaves of Trypilska and Holikovska cultivars. Thus, the combined effect of PEG and NPs restored the water balance in these cultivars to 89% and 87%, respectively. The restoration of RWC with NPs treatment of the third experimental genotype Favorytka was not detected.

RWC may be attributed to differences in the ability to absorb more water from the soil and/or the ability to control water loss through stomata. Plants react to water deficit with a rapid closure of stomata to avoid further water loss via transpiration (Gago et al., 2016). An adaptive way for plants to maintain leaf turgor under stressful conditions is an osmotic adjustment (Blum, 2005). Under drought conditions, plants accumulate organic compounds – osmolytes, which contribute to osmotic adjustment (Barbieri, 2011). Previous studies showed the resilience-anisohydric strategy of investigated cultivars for water balance regulation. In particular, they provide an osmotic adjustment and can retain a high level of organic solutes with osmoprotective properties under PEG-induced drought stress (Smirnov et al., 2020b). PEG with high molecular weight has been long used as a non-penetrating non-ionic inert osmoticum lowering the water potential of nutrient medium without being taken up or being phytotoxic (Hassan et al., 2004).

In our investigation for inducing water deficit in wheat seedlings, PEG 6000 solution was used. Polyethylene glycol with molecular weight 6000 is a non-penetrating osmotic agent and is widely used for the simulation of water deficit. PEG decreases the water potential of the growth medium resulting in the blockage of water absorption by the root system (Meher et al., 2018).

Effects of water deficit on PSII photochemistry

PSII plays a major role in the transformation of radiant energy in chloroplasts; therefore, the parameters reflecting PSII efficiency are widely used for monitoring the activity of plants' photosynthetic apparatus. One of the main methods to investigate the function of PSII and its reactions to environmental changes together with growth conditions could be quantified by chlorophyll a fluorescence measurement. It is an indirect diagnostic technique, a non-invasive method to detect photosynthetic reactions in plants and the tolerance to environmental stressors. This technique analyzes the effects of abiotic stress factors on photosynthesis (Maxwell, 2000).

Chlorophyll a fluorescence measurement could be widely used to examine photosynthetic performance in leaves in a laboratory and controlled environment. It can provide useful information on physical changes in pigment-protein complex, excitation and energy transfer, primary photochemistry, and the operating quantum efficiency of electron transport through PSII (Govindjee, 1995; Baker, 2004). Chlorophyll fluorescence parameters from leaves of the three wheat cultivars, grown on PEG-medium, from pre-soaked seeds with colloidal solutions of NPs, and in a combination of both treatments (PEG and NPs) are presented in Table 1.

Parameter F_v/F_m is a potential maximal quantum yield of PSII photochemistry in dark-adapted leaves. It is used to estimate the PSII efficiency when the plastoquinone (PQ) pool is fully oxidized and characterizes the content of photochemically active centers of PSII, which depend on the processes of destruction and the reparation of PSII. It should be noted that seed treatment by metal nanoparticles did not cause any changes in F_v/F_m in

cv. Trypilska and Holikovska except to Favorytka. The increase of F_v/F_m value in the latest cv. is characterized by a higher amount of active PSII centers. They are able to carry out photochemical reactions. PEG-simulated water deficit induced the decrease of F_v/F_m in leaves of two wheat cultivars which can be associated with the PSII damage. The results of this study show that the combined action of PEG and NPs reduced the negative effect of drought on seedling, as evidenced by F_v/F_m enhance in all wheat cultivars.

Effective quantum yield of PSII, F_v'/F_m' , is used to evaluate the maximum efficiency of PSII photochemistry at a given actinic irradiance when the PQ pool is partly reduced. The value of F_v'/F_m' was not significantly changed between treatments.

Photochemical quenching (qP) is related to a proportion of open PSII centers at certain conditions and characterized the degree of QA pool oxidation. It depends on both the inflow of electrons to QA and their outflow to the PQ pool. No significant difference in qP after

Table 1. Chlorophyll fluorescence induction parameters of wheat leaves under pre-soaked application with metal nanoparticles (NPs), grown with polyethylene glycol (PEG), and their combined action; n=10

Wheat cv.	Treatment	F_v/F_m	F_v'/F_m'	qP	qN	ϕ PSII
TRYPILSKA	Control	0.781 ± 0.007	0.599 ± 0.024	0.758 ± 0.036	0.561 ± 0.067	0.456 ± 0.039
	NPs	0.771 ± 0.004	0.596 ± 0.025	0.713 ± 0.048	0.571 ± 0.055	0.427 ± 0.043
	PEG	0.738 ± 0.009	0.594 ± 0.02	0.733 ± 0.032	0.448 ± 0.017	0.437 ± 0.034
	PEG and NPs	0.757 ± 0.002	0.602 ± 0.002	0.730 ± 0.007	0.528 ± 0.007	0.439 ± 0.006
HOLIKOVSKA	Control	0.773 ± 0.007	0.584 ± 0.020	0.667 ± 0.029	0.606 ± 0.011	0.366 ± 0.019
	NPs	0.779 ± 0.001	0.624 ± 0.031	0.674 ± 0.048	0.513 ± 0.063	0.422 ± 0.052
	PEG	0.756 ± 0.008	0.571 ± 0.020	0.538 ± 0.044	0.542 ± 0.069	0.307 ± 0.014
	PEG and NPs	0.771 ± 0.004	0.617 ± 0.027	0.635 ± 0.018	0.503 ± 0.011	0.321 ± 0.017
FAVORYTKA	Control	0.748 ± 0.005	0.619 ± 0.024	0.741 ± 0.022	0.409 ± 0.059	0.459 ± 0.031
	NPs	0.769 ± 0.006	0.604 ± 0.018	0.731 ± 0.015	0.524 ± 0.028	0.441 ± 0.02
	PEG	0.723 ± 0.013	0.568 ± 0.014	0.467 ± 0.081	0.463 ± 0.029	0.267 ± 0.053
	PEG and NPs	0.745 ± 0.013	0.588 ± 0.035	0.664 ± 0.081	0.485 ± 0.024	0.395 ± 0.067

F_v/F_m – maximum quantum efficiency of PSII photochemistry; F_v'/F_m' – maximum efficiency of PSII photochemistry in the light, if all centres were open; qP – photochemical quenching: relates PSII maximum efficiency to operating efficiency, non-linearly related to proportion of PSII centres that are open; qN – non-photochemical quenching coefficient; ϕ PSII – PSII operating efficiency: the quantum efficiency of PSII electron transport in the light

the treatment of seed with NPs colloidal solutions was observed. At the same time, PEG induced the lowering of qP in comparison to control in all wheat cultivars. Decreasing the qP value under water deficit is related with slower QA pool oxidation as a result of reducing the outflow electron rate to photosystem I (PSI). The highest reduction of these parameters (0.467) was observed in Favorytka. This can be explained by the dropping of qP below 0.6 due to the development of photoinhibition, which is determined as a light-dependent reduction of photosynthesis (Aro, 1993). However, the combination of PEG and NPs leads to recovery of qP.

The process of non-photochemical quenching qN (thermal dissipation of energy) is the main mechanism of regulation functional size in PSII antenna and protection for the photosynthetic apparatus against photooxidative damage (Stroch, 2004). There was a higher level of qN in seedlings leaves grown with PEG under the activation of photochemical reactions by 200 $\mu\text{mol (quantum)}/\text{M}^2\text{s}$.

The parameter ϕPSII was used to determine the real quantum yield of linear electron transport through PSII. We have found that the tendency of ϕPSII changes was similar with to the qP parameter. It was noticed that the PEG application induced the reduction of ϕPSII . Nevertheless, the seed treatment by NPs under water deficit causes the recovery of ϕPSII .

Taken together, these data indicate a more pronounced decrease in qP, electron transport capacity and an increase in dissipation of absorbed photon energy of cv. Favorytka.

Effects of water deficit on pigment content

The pre-soaked treatment with NPs solution led to an increase of chlorophyll a content in all experimental cultivars. The same tendency was observed for the content of chlorophyll b in Trypilska and Holikovska cultivars under NPs treatment. Plants responded to water deficit by the pigment composition changes of photosynthetic apparatus (PSA). The effect of water deficit modelling by PEG triggered chlorophyll a and chlorophyll b degradation in seedlings of Favorytka cultivar. The combined action

of PEG and NPs did not facilitate the recovery of the green pigment pool in this cv. The green pigment pool in Trypilska and Holikovska genotypes was unchanged, which contributes to PSA structural stability (Figure 2, A, B).

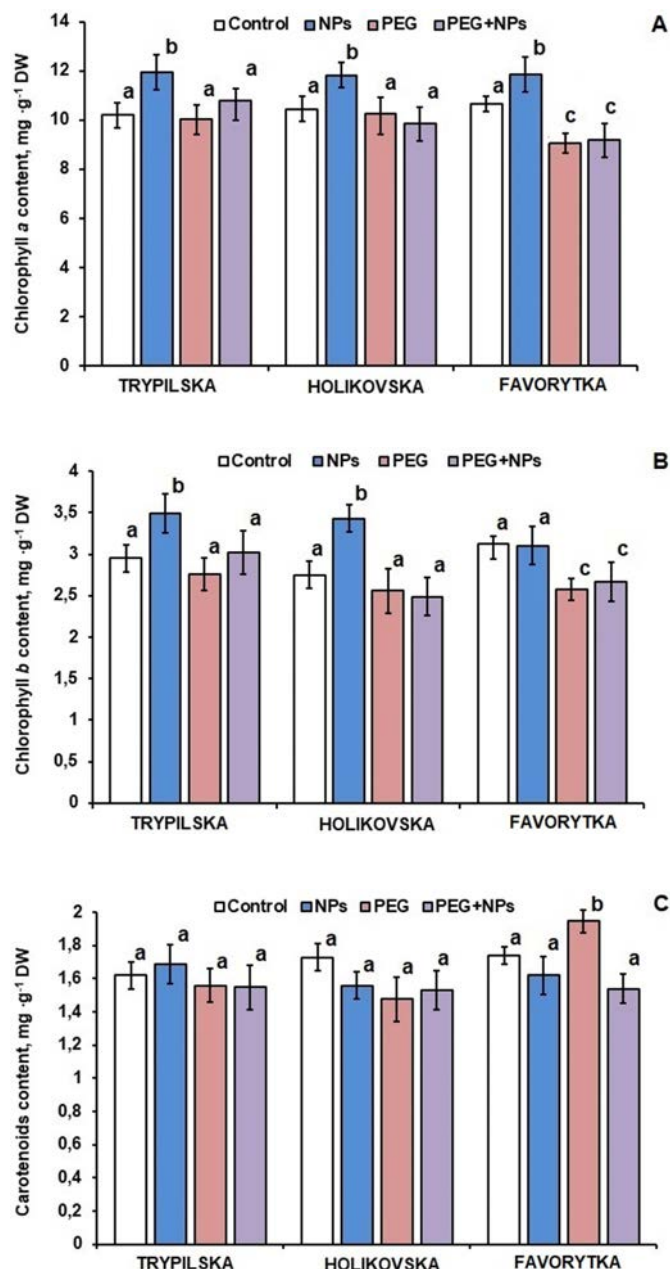


Figure 2. The content of photosynthetic pigments in wheat leaves under polyethylene glycol (PEG), metal nanoparticles (NPs) and their combined action; n=10 (means followed by the same letters were not significantly different at $P < 0.05$ according to the Duncan's multiple range test)

Protection of photosystem II (PSII) from reactive oxygen species, formed under stressors action including water deficit, is essential to avoid damage to the photosynthetic apparatus. Carotenoids are known to play a crucial role in these processes based on their property to deactivate triplet chlorophyll and singlet oxygen and protect the reaction centres of PSA from photooxidation (Jahns and Holzwarth, 2012). PEG-modelling water deficit, NPs and their combined action did not cause any significant changes in carotenoids content in seedlings of Trypilska and Holikovska genotypes (Figure 2, C). It was shown that water deficit simulated by PEG increased the concentration of total carotenoids in the Favorytka genotype. The induction of carotenoids synthesis can be related to the scavenging of reactive oxygen species and protection of PSA reaction centres from photooxidation in this genotype. The stable level of total carotenoids in seedlings of Trypilska and Holikovska genotypes on the background of chlorophyll a fluorescence measurement and stable content of chlorophyll a and chlorophyll b can testify to the greater stability of these cultivars to the effect of PEG-modelling water deficit.

CONCLUSIONS

All wheat cultivars showed diversity in their ability to tolerate chemical dehydration induced by PEG and perceived a decrease in osmotic potential as moderate stress. The results of photosynthetic pigment composition and chlorophyll fluorescence induction parameters showed that Trypilska and Holikovska cultivars were more tolerant to water deficit, in contrast to the more sensitive Favorytka cultivar. Pre-soaking treatment of seeds by NPs did not bear additional pressure on the seedlings of investigated wheat cultivars grown under polyethylene glycol-induced water stress. Nonetheless, the NPs solution did not decrease the negative effect of the simulated water deficit in the cv. Favorytka seedlings.

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