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EFFECT OF *CHENOPODIUM QUINOA* SEED EXTRACT IN PROTECTING THE THYLAKOID MEMBRANE UNDER THE INFLUENCE OF Cd and Na SALTS

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Abstract. The antioxidant properties of an extract obtained from quinoa (*Chenopodium quinoa*) seeds were determined by restoring the activity of Photosystem II (PSII) exposed to oxidative stress caused by the action of toxic metal salts. Studies were carried out on leaves of wheat seedlings (*Triticum aestivum*) incubated in a solution of Cd and Na salts with toxic concentrations using the delayed fluorescence method (msDF Chl **a**). It was revealed that electron transfer was blocked at P680*- Q_A-Q_B site into the electron transfer chain in PSII. Under these conditions, the extract exhibited a correlating effect in restoring PSII activity, which correlated with the control experiment on quenching the DPPH free radical with quinoa extract. The antioxidant activity of the extract is due to the presence in the phytochemical composition of quinoa seeds of low molecular weight compounds capable of extinguishing free radicals that cause damage to the thylakoid membrane.

Keywords: *Photosystem II, oxidative stress, quinoa, antioxidant effect, DPPH.*

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1. Introduction

Plant response to stress caused by reactive oxygen species triggers protective mechanisms. The plant organism has various effective defense systems against factors that cause oxidative stress (Sharma *et al*., 2020; Renger & Renger, 2008; Jafarova *et al*., 2021). The damaging effect of ROS is counteracted by the antioxidant defense system which along with the removing of free oxygen eliminates compounds damaged as a result of spontaneous oxidation by oxygen. The main role in this reaction belongs to antioxidant enzymes and low molecular weight compounds - carotenoids, ascorbic acid, glutathione, α-tocopherol, flavonoids, etc. Detailed research has been conducted into the antioxidant properties of plants that are potential sources of natural antioxidants (Ganiyeva *et al*., 2009; Jafarova *et al*., 2014). One of them is the species *Chenopodium quinoa* exhibited high antioxidant activity caused by wide range of phenolic compounds in its chemical composition including flavonoids and anthocyanins (Abugoch *et al*., 2009; Zhu *et al*., 2001). The accumulation of ROS leads to significant damage of biological processes in the cell and particularly in chloroplasts (Järvi *et al*., 2015; Daisuke *et al*., 2016). Chloroplasts are the main organ producing ROS and the most vulnerable is PSII and its

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oxygen evolving complex (Ahmad *et al*., 2008; Barber, 2003; Foyer, 2018). Its destruction under stress conditions leads to denaturation, proteolysis of proteins and lipid peroxidation in the reaction center (RC) and suppression of electron transfer within electron transport chain (Roach & Krieger-Liszkay, 2014; Han *et al*., 2019; Foyer & Noctor, 2005). Photosynthetic efficiency is suppressed by the action of metals in toxic concentrations on plants. As has been confirmed by many researchers, ions of toxic metals are integrated into the electron transport chain (ETC) and affect its activity (Gaziyev *et al*., 2011). When electron transport exceeds the needs of normal metabolism, molecular oxygen is reduced to reactive forms, causing an oxidative explosion. The target of ROS is the D_1 protein, which performs the primary functions of charge separation and electron release from the chlorophyll reaction center PSII (Järvi & Aro, 2021). It is known that in nature prolonged exposure to stress factors causes a nonspecific increase in plant resistance called cross-adaptation. Perhaps this phenomenon is associated with the increased activity of protective antioxidant high-molecular and low-molecular compounds capable of neutralizing free radicals. Based on the above, the task was set to determine the ability of an extract from *Chenopodium quinoa* seeds to restore the activity of the electron transport chain of PSII, which is suppressed under salinity conditions.

2. Material and methods

In the experiments we used 7-day-old wheat seedlings (*Triticum aestivum L*.) grown in an aquatic environment at +24 C, humidity 80% and illumination 250 μW/cm^2. The seedlings were transferred to solutions containing high concentrations of Cd and Na salts (10^{-3} M) for 24 hours in order to create an increased content of reactive molecules in the chloroplasts. Stressed seedlings were transferred to an extract solution (2:3) for the same time in order to determine it as an antioxidant. The extract from *Chenopodium quinoa* seeds was obtained by extraction with a 40% ethanol solution in water for 14 days with stirring. The antioxidant activity (AO) of the extract was determined by the DPPH quenching method (Brand-Williams *et al*., 1995). The optical absorbance of the methanolic solution of DPPH at 518 nm was adjusted to 0.45, which corresponded to a concentration of 40 μM. Time resolved absorption changes were measured at peak maximum λ =518 nm for 20 minutes on a UV-Vis spectrophotometer (Jenway 7305). Concentrations were calculated from a calibration curve ranging from 1 to 10 μM Trolox. All measurements were carried out in a cuvette with an optical path of 10 mm. The functional state of the seedlings was assessed based on changes in the characteristics of millisecond delayed fluorescence (ms DF Chl **a**) reflecting partial reactions of the electron transport chain within PSII of chloroplasts. The delayed fluorescence measurements were carried out on a fluorimeter equipped by phosphoroscope with a time resolution 1.25 ms (Goltsev *et al*., 2009).

3. Results and discussion

The activity of photochemical processes in the reaction center (RC) of PSII under the action of Cd and Na salts on seedlings was assessed by changes in the kinetic curves of ms DF Chl **a**. Analysis of fluorescent characteristics showed a decrease in the ratio of fast (f) and slow (sl) fluorescence phases to the stationary level of the induction curve (st) which determine the activity of the donor (f/sl) and acceptor (sl/st) side of the electron transport chain (ETC) of PSII (Fig. 1, 2). The quinoa seed extract was used as a protection

agent against oxidative stress caused by the Na and Cd ions and the effect of antioxidant activity was determined (Table 1).

Table 1. Comparison of DPPH quenching by Trolox and *Chnopotium guinoa* extract

Samples	**IC_{50} (µg/ml)***
Trolox	17.1±0.1
Extract of ***Chenopodium quinoa***	19±0.1

When the extract acted on wheat seedlings incubated in a solution containing Cd salt for 24 hours these values increased by only 33% and 13% relative to the action of Cd ions (Figure 1). When the seedlings were incubated in a solution containing the extract for 24 hours and then transferred to the solution contained Cd ions, a sharp increase in the f/sl and sl/st ratio was observed by 76% and 71% respectively, compare to the action of Cd ions (Figure 1). The action of the extract strongly suppressed the toxic effect of the metal in second variant and the activity of the ETC exceeded the control (Figure 1).

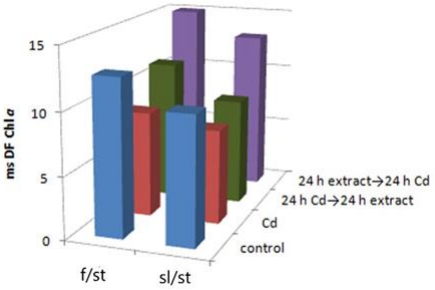

Figure 1. Changes in the ratio of fast and slow fluorescence to stationary fluorescence (f/st, sl/st) characterizing the operation of the PSII ETC under the conditions of: variant 1 – 24 h Cd^{2+} → 24 h extract; variant 2 – 24 h extract → 24 h Cd^{2+}.

Addition of quinoa extract had another effect in the presence of NaCl compare to experiments with Cd ions. When the seedlings were incubated in a solution with the extract for 24 hours and then in a salinity environment for the same time, the fluorescent characteristics, especially Fast phase fluorescence showed a slight increase in the activity of the ETC relative to the action of salt. The value of f/st increased by only 0.7% and the value of s/st - by 23% (Figure 2). In seedlings incubated in a NaCl salt solution and transferred to a medium containing the extract fast fluorescence activity increased by 31% and slow fluorescence activity increased by 33%, relative to the inhibitory effect of NaCl (Figure 2).

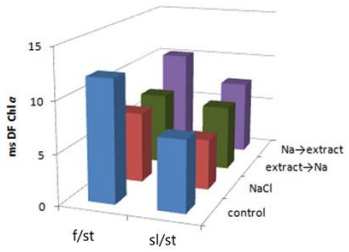

Figure 2. Changes in the ratio of fast and slow fluorescence to stationary fluorescence (f/st, sl/st) characterizing the operation of the PSII ETC under the conditions of: variant 1 – 24 h extract → 24 h NaCl ; variant 2 – 24 h NaCl→ 24 h extract.

ROS generated during the toxic action of salts suppress photochemical reactions in the ETC of PSII. Both the donor and acceptor sides are inactivated as a result of disruption of electron transfer in the P680*- Q_A-Q_B site of the PSII ETC which is detected by changes in fluorescent characteristics (ms DF Chl **a**) (Gasanov *et al*., 2015; Gasanov *et al*., 2007). Suppression of PSII activity by the toxic effects of Cd and Na salts correlates with the addition of quinoa extract. This confirms the AO activity of quinoa seed extract detected by the DPPH method. This implies that quinoa extract has a protective or mitigating effect on the toxic impact of Cd and Na salts on PSII activity, potentially indicating its ability to counteract or reduce the negative effects of these toxic salts on the photosynthetic process in plants. It has been shown that the effect of quinoa extract depends on the experimental conditions and the active metal. The positive effect of quinoa extract on the resistance of PSII to oxidative stress caused by $CdCl_2$ and NaCl is due to the phytochemical composition of quinoa seeds, rich in flavonoids, phenolic compounds, vitamins and proteins that can neutralize ROS formed during stress thereby maintaining the redox balance between systems.

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