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PROTECTION OF PHOTOSYNTHETIC PIGMENTS FROM TOXIC ACTION OF COPPER BY PLANT EXTRACTS

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Abstract. The effect of 10^{-3}M concentration of copper on photosynthetic pigments chlorophyll a and b and carotenoids contents in 7-days seedlings of wheat plants (*Triticum aestivum* L.) was studied. The copper action was shown to decreased the chlorophyll a and chlorophyll b and carotenoids in leaves. To protect from toxic action of Cu^{2+} the extracts of sage leaves (*Folia Salvia officinalis*), roots of liquorice (*Radix glycyrrhizae*) and leaves of Danae racemosa that was shown are rich by an antioxidant properties were used. The greatest protective role of chlorophyll a and b forms was obtained to have roots of liquorice that contain saponins and more than 30 flavonoids, that have the capacity to quench oxidative damage and scavenging active oxygen radicals. Besides, the protection of photosynthetic pigments was found to have extracts of sage leaves and Danae racemosa, containing flavonoids and carotenoids.

Keywords: *Chlorophyll a and b, carotenoids, oxidative stress, plant extracts.*

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1. Introduction

Biosynthesis of photosynthetic pigments is a process prone to several biotic and abiotic stresses including metal stress. Inhibition of photosynthetic pigment biosynthesis is one of the primary events in plant during metal stress (Krupa *et al*., 1993). As a consequence a delay in the assembly of photosynthetic apparatus, lower photosynthetic efficiency, slower plant growth and decreased biomass production occurs (Mysliva-Kurdziel & Strzalka, 2002). The biological phytotoxicity and availability of heavy metals is controlled and is dependent upon a number of factors including the elements uptake site, competition for binding sites, irradiance and pH of medium (Gaziyev *et al*., 2011; Ganiyeva *et al*., 2013).

Several metals are essentials for plant development and life. Many bio-molecules and reaction in plant require essential elements, Fe for electron-transporters and chlorophyll (Chl biosynthesis, Mg for functional Chl, Cu for plastocyanin). Usually most of the vital elements are present in a concentration range which is compatible with life. When metals are present in excess, they become toxic. One mechanism leading to inhibition of photosynthesis by heavy metals is their substitution for Mg^{2+} in the Chl molecules (Solymosi *et al*., 2004), leading to a breakdown in photosynthesis. In case of metal pollution, the change in ratio Chl a and b suggests that the metal differentially affects the light-harvesting complex (LHC 2 of PS II) were Chl b is located, rather than the Chl a reaction centers (Wisniewski & Dickinson, 2003; Aravind & Prasad, 2004). Excess Cu^{2+} may replace other metals in metalloproteins or may interact directly with SH groups of proteins. Cu-induced free radical formation may also cause protein damage (Weckx & Clijsters, 1996). High concentration of Cu^{2+} may catalyze the formation of hydroxyl radical from O_2 and H_2O_2. Excess copper can catalyze the generation of harmful reactive oxygen species (ROS) such as singlet oxygen ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) which can damage biological molecules by inducing lipid peroxidation. This Cu^{2+}- catalyzed reaction takes places mainly in chloroplasts. The hydroxyl radical may start the peroxidation of unsaturated membrane lipids and chlorophyll and these inhibitory mechanisms might contribute to the observed inhibition of electron transport by Cu^{2+} (Clijsters & Van Assche, 1985). Both the donor and acceptor sides of PS II have been proposed to be most sensitive site for Cu^{2+} action (Yruela *et al*., 1996; Jegerschöld *et al*., 1995). On the donor side Cu^{2+} is thought to inhibit electron transport to P680, the primary donor of PS II (Schröder *et al*., 1994). In addition, the redox active Cu ion induced lipid peroxidation (Shainberg *et al*., 2001) that further results in serious damages of thylakoids, namely disintegration of lamellar system and increased unstacking of thylakoids. The excess Cu also decreases the photosynthetic pigments affecting both their synthesis and degradation. The established lower content of trans hexadecenoic may decrease the oligomerization. Thus the decreased C 16:1t fatty acid content may diminish the content of LHC 2 oligomer that means less efficient energy collection and distribution between photosystems.

The use of traditional medicine is widespread and plant still present a large source of natural antioxidants (Miliaskas *et al*., 2004).

Plants have evolved protective enzymatic and nonenzymatic mechanisms to scavenge ROS. Molecules such as glutathione, ascorbate and carotenoids provide nonenzymatic protection (Scandalios, 1997; Smolikova et al., 2015).

In the present work the plant extracts from Azerbaijan flora rich by antioxidants such as leaves of sage (*Folia Salvia officinalis*) roots of liquorice (*Radix glycyrrhizae*) and leaves of relict plant Danae racemosa were used as protectors.

2. Material and methods

The seven days wheat plants (*Triticum aestivum* L.), sort Caucasus were object of investigations. The plants were grown in water medium and irradiated with white light (250 Wm^2 S^{-1}), 22°C humidity 80%. After 7 days the plants were located on 48 hours to $CuSO_4$ (10^{-3}M) solution and then to extracts of tested plants (5 µg/ml) at different pH of medium (4,5; 6,8; 9,0). The chlorophyll (Chl) absorbance spectra were registrated on spectrophotometer Furye Cary 50 Scan Varian. The chlorophyll content was determined by absorption value at wavelengh: for Chl a – 663 nm, 680 nm and Chl b – 645 nm, carotenoids: at 440 and 480 nm. The scrining of alcoholic extracts of dried plant: sage leaves (*Folia Salvia officinalis*) with predominant content of saponins (ursolic oleanine acid, carotene, vitamine C (Kerimov, 1982), roots of liquorice (*Radix glycyrrhizae*) with saponine-glycyrrhizin (Damirov *et al*., 1982) and leaves of Danae racemosa with predominant content of carotenoids (Kerimov, 1982; Dadasheva *et al*., 1990) was performed. The extraction of crumble material was undataken with 95% ethanol during 7 days. The average devitations were calculated on the base of 3 analyses.

The statistical treatment of data was worked out according to Excell programme.

3. Results and discussion

The toxic action of Cu^{2+} on photosynthetic pigments: carotenoids (440 and 480 nm) and chlorophylls (645 nm, Chl b), chlorophyll a (663 and 680 nm) as was obtained to decreased of all pigments content at high concentration of Cu^{2+} in medium (10^{-3}M). The obtained data on absorption of pigments at stress situation as compared with control taking (to 100%) compose at acid medium under 440 nm – 64%, at 480 nm – 63%, at neutral medium at 440 nm – 52%, 480 nm – 47% (Fig. 1).

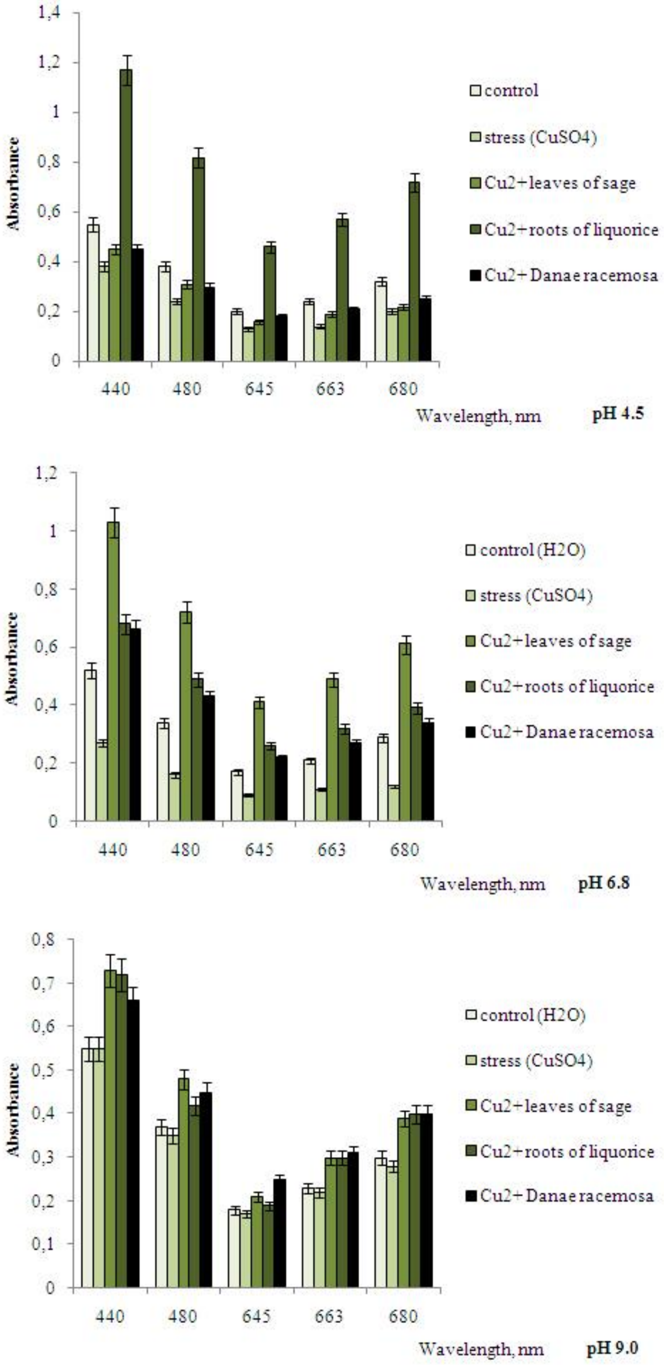

Fig. 1. Action of Cu^{2+} ions on photosynthetic pigments forms Chl a, Chl b and carotenoids and protecting effect by plant extracts from leaves of sage (*Folia Salvia officinalis*) roots of liquorice (*Radix glycyrrhizae*) and leaves of relict plant Danae racemosa

Under indicated conditions of stress Chl b as compared with control was decreased by up 35%, the Chl a (663 nm) – up to 52% and under 680 nm – on 37%.

Under medium pH 6,8 Chl b under stress as compared with control decreased up to 50%, Chl a (663 nm) – to 48%. The Chl a 680 nm on 59%. The stress situation was not observed under alkaline condition.

So, heavy metals exposure caused decrease in Chl and carotenoids content there protection has prompted to search for compounds with potent antioxidant activity (Ganiyeva *et al.*, 2009; 2018). The decreased content of chloroplastic pigments may be an outcome of reduced synthesis and/or enhanced oxidative degradation of pigments by the imposed oxidative stress.

As Fig. 1 indicates the inhibition of photosynthetic pigments may be overcome by adding of protectors, namely extract of liquorice demonstrated increase of all photosynthetic pigments up to 2 times. Extract of sage leaves (*Folia Salvia officinalis*) under pH 6,8 increased all pigments up to 2 and more times (Fig. 1).

The increase of carotenoids level as it is in Fig. 2 was degisned. Extracts of sage and Danae racemosa in acid pH (4,5) protected of pigments supressed by Cu^{2+} toxic action. Effect of sage is observed at absorbtion region 480 nm and 663 nm and was by up to 29% and 36% higher as compared with action of Cu^{2+}. Effect of Danae racemosa exceeds of effect of sage at 480 nm, 645 nm, 663 nm, 680 nm relatively action of Cu^{2+} by up to 25%, 38%, 50% and 25%. The liquorice protect of pigments more than by up to 100%. Effect of liquorice (Fig. 2) is likely to be due to that in its composition besides of vitamin C and saponins glycyrrhizin presents of flavonoides (licuraside predominant flavonoid of this plant). At alkaline medium the toxic action of Cu^{2+} and protective posibilities of extracts was not observed probably due to lowering of solubility of copper salts (Putilina, 2009).

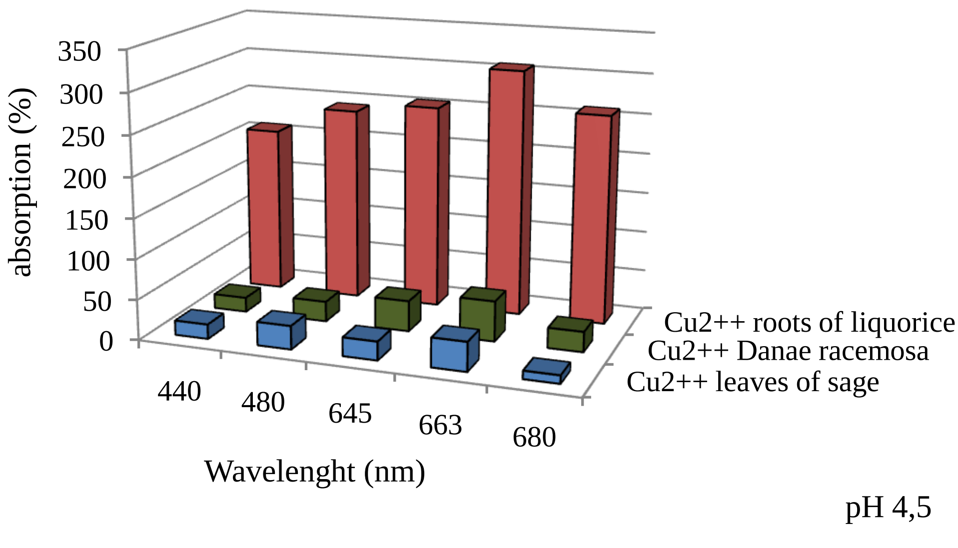

Fig. 2. The pigments absorption restoration relatively to stress induced of Cu^{2+} by leaves of sage, roots of liquorice and Danae racemosa extracts at pH 4,5, expressed in percentage

The carotenoids accumulation is often regarded as one of the mechanisms to counteract stress in organisms (Mallick & Mohn, 2000). As triplet state quenchers, carotenoids are able to protect the photosynthetic apparatus by quencing higly reactive chlorophyll triplet states as soon as they are formed. Enhanced synthesis of Car by free radicals is repoted (Schroeder & Johnson, 1995b) under Cu^{2+} stress.

The Car are known to be potent quencher of ROS, particulary singlet oxygen species (Middleton & Teramura, 1993). The efficacy of Car in protecting of the photosystems is likely due to their function as efficient quencher of high-energy shotwave radiation (Demmig-Adams, 1990). The mechanism by wich this is accomplished was first proposed to involve a photochemical state change of singlet oxygen to triplet form by interaction with carotenoids, removing the potentially dangerous oxygen radicals produced in photooxidative processes (Krinsky, 1979). Functionally, the carotenoids, expecially xantophylls, absorb the shortest wavelengh radiation within the light-harvesting complexes.

It is well known that carotenoids can act as antioxidants by quenching singlet oxygen or photosensitizer triplet states and that this protective role of carotenoids is important in photosynthesis. The quenching of singlet oxygen (${}^1O_2^*$) by carotenoids and the mechanism by which this reaction protects against ${}^1O_2^*$ mediated have been much discussed.

The activity of carotenoids to quench Chl excited states suggested that carotenoids may play a role in regulating energy flow in photosynthetic antennae. One of the nonphotochemical routs is linked the formation a transthylakoid pH gradient which drives the enzymatic epoxidation and deepoxidation of carotenoids associated with PS II. This reversible process known as xantophyll cycle has been implicated in the protection of photosynthetic systems. The process depends on the presence of both an acidified lumen and deepoxidated xantophylls. Induction of an acid lumen pH is the basic requirement for the operation of xantophyll cycle. The enzyme of violaxanthin deepoxidase is responsible for converting violaxanthin to reaxanthin at acid lumen pH (Choudhury & Behera, 2001). The xantophylls lower ${}^3Chl^*$ formation by quenching excited singlet state Chl (${}^1Chl^*$) (Siefermann-Harms, 1987). Carotenoids protect photosynthetic apparatus through two important ways: (β-carotene (β-Car) directly quenches both triplet chlorophyll (${}^3Chl^*$) and singlet oxygen (${}^1O_2^*$).

Thus, decisive protective role is obtained to have a roots of liquorice that contained saponins, that is known to bind with free radicals and lead out from organisms and more than 30 flavonoids. Moreover, the protection of photosynthetic pigments was found to have extracts of sage leaves and Danae racemosa, containing flavonoids and carotenoids (Pietta, 2000).

The used plant extracts rich by biologically active compounds might offer protection to photosynthetic membrane from oxidative damage.

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