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Physiological reaction of basket willow (*Salix viminalis* L.) to copper excess in hydroponic medium

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Abstract

The influence of copper salt, in a concentration range of 50–150 mg·dm^{-3}, on the physiological response of basket willow was studied on Tora variety, which were grown in hydroponics. Content of assimilation pigments, relative water content (RWC), water saturation deficit (WSD), intensity of assimilation and transpiration, stomatal conductance, photosynthetic rate of water use efficiency (WUE) and instantaneous photosynthetic rate of water use (WUEI) were determined in leaves. It was observed that the decrease in the concentration of tested physiological parameters correlated with the increase in copper salt doses in the medium. The assimilation and transpiration of basket willow were significantly limited by stomata. The addition of copper salt to the medium increased WSD in leaves of the basket willow. The obtained results of the studied physiological parameters may prove useful for the assessment of resistance of the studied willow cultivar to stress caused by increased copper ion content in medium and its applicability in reclamation of areas degraded by humans.

Key words

Salix viminalis L., copper stress, CO_2 assimilation, photosynthetic pigments, transpiration, water content, water use efficiency

Introduction

Environmental pollution requires undertaking intensive treatments aimed at restoring the natural environment to its original condition. Heavy metals are amongst the substances that have a negative influence on the environment. Copper is one of such substances; it is an element that is necessary for the plant's life and, at the same time, strongly toxic if it occurs in excess in the environment. It activates a lot of enzymes in a plant and a component of catechol oxidase, ascorbate oxidase, superoxide dismutase, plastocyanin and copper flavoproteins (Szatanik-Kloc et al. 2010). In addition, it participates in the processes of photosynthesis, respiration and protein formation and in the transformation of nitrogen compounds and carbohydrates. It participates in the metabolism of cell membranes and influences their permeability and also the water balance. Copper belongs to a group of met-

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als that have the ability to catalyse the Fenton-catalysed Haber–Weiss reaction and its excess can directly contribute to an increase in the concentrations of reactive oxygen species in cells (Kehrer 2000). Copper toxicity involves the reduction of the most important vital processes in plants both at the physiological and biochemical levels (Groppa et al. 2001; Wang et al. 2004) and inhibits root growth (Gajewska and Skłodowska 2010).

The effectiveness and duration of remediation using phytoremediation does not only depend on the concentration of pollutants in the soil but also depend on the proper selection of plants for a given type of pollution (Zemleduch and Tomaszewska 2007). Research conducted in the area of chemistry, physiology and biochemistry allowed the acquisition of more accurate knowledge on the mechanisms and processes responsible for toxin collection and accumulation by plants and also on the use of physiological and biochemical parameters for the assessment of usefulness of plants in the remediation of degraded areas. In this way, it is possible to use the natural properties of plants and to plan optimal pollution remediation methods in a degraded area (Zemleduch and Tomaszewska 2007). Phytoremediation is a method of reclamation of areas contaminated with copper. From the point of view of phytoremediation of large industrial protection zones and urban areas, it is the plants that belong to the genus *Salix*, a large number of which are considered to be resistant to soil contamination with heavy metals, that seem to be particularly predisposed to do well under such conditions (Landberg and Greger 1996; Wrzosek et al. 2008). Physiological and biochemical parameters are used for the evaluation of plants useful in reclamation of degraded areas.

The study aimed at determining the physiological activity of basket willow cv. Tora to elevated copper ion content in medium. Willow verification as a genus of particular value for phytoremediation, understanding of physiological grounds of its resistance to stress caused by increased level of copper ions in medium, will enable for a preliminary determination of the usability of the cultivar for reclamation.

Material and methods

The biological material used in the experiment was basket willow cv. Tora, the seedlings of which originated from the certified stock plantation Hvidsted Energy Forest in Denmark. Sixty cuttings of willow obtained from the past year's shoots were used for the studies. Laboratory studies were carried out as a hydroponic culture with different doses of copper. Willow cuttings were divided into four groups of the same number and placed in containers filled with Hoagland full nutrient solution. Copper sulphate was added to the nutrient solutions 14 days after the cuttings were placed in the hydroponics when the plants had taken roots and had shot. Three levels of contamination of the nutrient solutions with copper salts were applied: 50, 100 and 150 mg dm^{-3}. Plants placed in Hoagland full nutrient solution were the control object. The determination of physiological parameters was carried out three times: on the 7th, 14th and 21st day after the day the rates of copper salts were applied. The measurements of the parameters of gas exchange, CO_2 assimilation (A), transpiration (E), stomatal conductance for water vapour (gs) and stomatal conductance for CO_2 (gc), were made (the measurement was replicated three times) on leaves using a gas analyser TPS-2, working in an open system with a chamber of PLC-4 type. In the analyser cuvette, the following conditions were established: a permanent inflow of carbon dioxide, humidity equal to the humidity of the environment and lighting equal to 2,053 PAR (μmol m^{-2} s^{-1}), supplied by means of a light unit attached to the cuvette. On the basis of the results obtained from the assimilation and transpiration rates, photosynthetic effectiveness of the use of water (WUE) and instantaneous photosynthetic index of water use efficiency (WUEI) were calculated. The determination of assimilation of pigments (chlorophyll 'a' and 'b' and carotenoids) was carried out on the same leaves on which the parameters of gas exchange were determined. The content of chlorophyll was defined using the method of Arnon et al. (1956) modified by Lichtenthaler (1987), whereas the content of carotenoids was determined by means of the Hager and Meyer-Bethenrath (1966) method. The indices of the relative water content (RWC) and the deficit of water saturation (WSD) were determined according to Bandurska (1991).

Statistical analysis

The statistical analysis of the studies was carried out using Duncan's test at the level of significance $LSD_{0.05}$. Pearson linear correlation coefficients between the analysed gas exchange parameters were also determined.

Results and discussion

On the basis of the results obtained, it can be determined that stress caused by the applied copper salt dosages influenced the change in examined physiological parameters of the tested cultivar of basket willow.

It was determined that increasing concentration of copper salt in medium resulted in a reduction of the content of photosynthetic pigments in the leaves of basket willow cv. Tora.

The highest applied concentration of copper salt resulted in reduction of chlorophyll 'a' and 'b' by approximately 40% relative to control plants. The carotenoids content in the leaves of the tested basket willow leaves at the applied copper salt dose of 150 mg dm^{-3} was 59.6% of the pigment content in leaves of the control plant. On the other hand, Cu salt doses of 50 and 100 mg dm^{-3} resulted in a decrease in the determined pigments by approximately 20% (carotenoids at a Cu salt dose of 50 mg dm^{-3}) to 41% (chlorophyll a at a Cu salt dose of 100 mg dm^{-3}; Tab. 1).

Malinowska and Wróbel (2015) Smolik and Malinowska (2009) also reported a decrease in the content of chlorophyll 'a' and 'b' in plants under the influence of large doses of copper. When excessive amounts of copper occur in the soil, this element is accumulated in large quantities in the roots.

In studies of Jurkowska et al. (1996), McBride and Martinem (2000), it was also demonstrated that Cu transport to the above-ground parts of the plants is slow because of the occurrence of a strong conductance barrier for this element from the roots to the stem. Inhibition of chlorophyll synthesis is a symptom of the impact of various heavy metals, which also includes excess copper in the plant. According to Stiborova et al. (1986), an excessive Cu content in the plant inhibits synthesis of especially chlorophyll 'a' and 'b' and reduces their content in photosynthetic cells. According to Szatanik-Kloc et al. (2010), copper ions are accumulated in chloroplasts if this element occurs in high concentrations, which interferes with the synthesis of photosynthetic pigments and enzyme activity.

A decrease in the intensity of net photosynthesis and transpiration was observed with an increase in the copper salt dose in medium. Intensity of the CO_2 assimilation process in the Tora cultivar in the presence of the highest copper salt dose in medium was 76.8% relative to control plant (Tab. 2). The applied dose of 150 mg dm^{-3} of copper salt resulted in a significant decrease in transpiration process intensity by 60.4% relative to the control plant in the tested willow cultivar (Tab. 2).

Similar reactions of plants to copper salt were observed by Malinowska and Wróbel (2015) and Smolik and Malinowska (2009). A decrease in the intensity of CO_2 assimilation and transpiration is probably caused by the toxic impact of copper on these processes. Excessive amounts of copper in the plant limit the process of photosynthesis by inhibiting the transport of electrons. In addition, copper influences the metabolism of cell membranes by increasing the K^+ ion secretion by exacerbating the water balance of plants and contributing to a decrease in the transpiration process (Ruszkowska and Wojcieska-Wyskupajtys 1996a,b; Maksymiec 1997;

Table 1. Mean values (± SD) of assimilation pigments and water balance indices in basket willow cv. Tora, depending on the applied copper salt dose. Percentage values in comparison to the control (100) are given in parentheses

Dose (mg dm^{-3})	Chlorophyll a (µg g^{-1} FW)	Chlorophyll b (µg g^{-1} FW)	Carotenoids (µg g^{-1} FW)	Relative water content (RWC) (%)	Water saturation deficit (WSD) (%)
0	1532 ± 198c (100)	645 ± 49 c (100)	842 ± 106 d (100)	89.1 c	10.9 a
50	1135 ± 110 b (74.1)	487 ± 56 b (75.5)	679 ± 97 c (80.6)	80.5 b	19.5 c
100	889 ± 85 a (58.0)	383 ± 52 a (59.4)	498 ± 88 a (59.1)	73.3 a	26.7 b
150	918 ± 91 a (59.9)	386 ± 58 a (59.8)	502 ± 76 b (59.6)	63.2 a	36.8 b

a–d represent homogeneous groups of every parameter according to the Duncan test, $\alpha < 0.05$.

Table 2. Mean values (±SD) of gas-exchange parameters and photosynthetic index of water use efficiency (WUE) and instantaneous photosynthetic index of water use efficiency (WUEI) in basket willow cv. Tora, depending on the applied copper salt dose. Percentage values in comparison to the control (100) are given in parentheses

Dose (mg dm^{-3})	CO_2 assimilation (µmol CO_2 ·m^{-2} · s^{-1})	Transpiration (mmol H_2O · m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	WUE (µmol CO_2 · mmol H_2O^{-1})	WUEI (µmol CO_2 · mmol H_2O^{-1})
0	7.79 ± 1.42 b (100)	1.92 ± 0.18 d (100)	120 ± 25 a (100)	4.06 a	0.065 a
50	7.03 ± 1.18 a (90.2)	1.19 ± 0.20 c (61.9)	110 ± 38 c (91.7)	5.91 b	0.064 a
100	6.44 ± 2.04 c (82.7)	0.97 ± 0.16 b (50.5)	98 ± 33 b (81.7)	6.64 c	0.066 a
150	5.98 ± 1.21 a (76.8)	0.76± 0.12 a (39.6)	85 ± 30 b (70.8)	7.87 d	0.070 b

a–d represent homogeneous groups of every parameter according to the Duncan test, α < 0.05.

Küpper et al. 2002). Szatanik-Kloc et al. (2010) thought that plants die in the presence of phytotoxic Cu^{2+} concentrations as a result of a reduced content of other micro- and macronutrients and related changes in plant metabolic processes.

Application of the highest copper dose in the tested cultivar resulted in a reduction of the stomatal conductance by 29% relative to the cultivar growing on full medium (Table 2).

Many authors have emphasised that stomatal conductance is regulated largely by carbon dioxide concentration in the intracellular spaces, leaf water potential and interaction of many environmental factors, amongst others, increased content of heavy metals in substrate (Maier-Maercker and Koch 1992; Mott and Buckley 2000; Tuzet et al. 2003).

The average values of water use rates for the examined basket willow cultivars ranged from 4.06 to 7.87 for WUE and from 0.064 to 0.070 for WUEI. Significant differences in the value of WUE and WUEI were obtained, depending on the copper salt dose used. In our studies, willow cultivar growing on medium with the highest copper salt dose was characterised by significantly higher values of the WUE coefficient (Tab. 2). According to Jeżowski et al. (2009), the photosynthetic of water use efficiency (WUE) coefficient is primarily determined by environmental conditions for plant growth, whereas photosynthetic intrinsic water use efficiency (WUEI) coefficient is determined by internal (genetic) traits of the plant. The calculated rate varied depending on the copper salt doses being used. Wróbel et al. (2006), Malinowska and Wróbel (2015) and Wróbel and Wróbel (2015) found in their studies conducted on the basket willow growing on a degraded substrate that cultivar Tora was characterised by a significantly higher WUE value. Jaroszewska et al. (2011) showed higher values of the photosynthetic rate of water use efficiency also for the cherry tree growing under water and nutrient stress conditions compared to the control conditions.

The RWC coefficient and the water saturation deficit (WSD) coefficients are, amongst other things, indicators of changes in the water balance.

The increasing copper salt doses resulted in a reduction of water content in leaves of the studied basket willow cultivar. The highest drop in the RWC by close to 26% was observed following the application of the highest copper salt dose in comparison to control plants (Tab. 1).

When exposed to stress, plants produce excessive amounts of osmoregulators and other substances. The changes observed in the physiological parameters tested may result from both stress induced by an increased number of copper ions in the medium and repair parameters (Wójcik and Tukendorf 1995; Starck 2002).

On the basis of the parameters of gas exchange, a linear correlation analysis between gas-exchange parameters was conducted. In the tested willow cultivar, significant positive relationships between assimilation, transpiration and stomatal conductance were observed. Analysis of correlation coefficients for the relationship between transpiration and stomatal conductance for water vapour exhibited the highest significant correlation coefficient in Tora at the highest applied Cu salt dose (Tab. 3).

Table 3. Values of coefficients of correlation between the parameters of gas exchange and stomatal conductance of basket willow cv. Tora

Dose of Cu (mg dm^{-3})	Parameters	Parameters	Correlation coefficients (r)
0	CO_2 assimilation	Stomatal conductance	0.419*
	Transpiration	Stomatal conductance	0.479*
	CO_2 assimilation	Sub-stomatal concentration of CO_2	0.296
50	CO_2 assimilation	Stomatal conductance	0.438*
	Transpiration	Stomatal conductance	0.573*
	CO_2 assimilation	Sub-stomatal concentration of CO_2	0.326
100	CO_2 assimilation	Stomatal conductance	0.594*
	Transpiration	Stomatal conductance	0.697*
	CO_2 assimilation	Sub-stomatal concentration of CO_2	0.529*
150	CO_2 assimilation	Stomatal conductance	0.572*
	Transpiration	Stomatal conductance	0.821*
	CO_2 assimilation	Sub-stomatal concentration of CO_2	0.676*

(r) * significant at $\alpha = 0.05$.

Assimilation and transpiration rates depend largely on stomatal conductance, which determines the rate of diffusion of water vapour out of the leaf and the linear velocity of forced air flow through the leaf (Mott and Buckley 2000). Also in Malinowska (2012) studies, significant linear correlations between assimilation and transpiration rates and stomatal conductance in the trees growing in the urban environment have been confirmed. Wróbel et al. (2006) have shown a linear correlation between transpiration and stomatal conductance in the basket willow growing under stress conditions. In the study conducted by Malinowska and Wróbel (2015), CO_2 assimilation and transpiration of the basket willow growing in a contaminated substrate have been significantly reduced by stomatal conductance. Stomatal closure is a response of plants to many stressors and follows a complicated mechanism that is associated with the phosphorylation of certain proteins and increased content of abscisic acid, which leads to H_2O_2 accumulation and activation of calcium channels in the membranes of guard cells, resulting in the reduction of transpiration rate (Laloi et al. 2004).

Reduction of the studied physiological parameters, including CO_2 assimilation and transpiration with the concomitant reduction of stomatal conductance in stress conditions, may be the trait that enables the studied willow cultivar to survive in relatively good shape under stress conditions and makes it particularly fit for reclamation.

The results obtained from the studied physiological parameters may prove useful for the assessment of resistance of the studied willow cultivar to stress caused by increased copper ion content in medium and its applicability in reclamation of areas degraded by humans.

Conclusions

1. The intensity of the CO_2 assimilation process and transpiration and the content of assimilation pigments in the studied basket willow cultivar varied because of the copper salt dose in the medium.
2. Net assimilation and transpiration of basket willow ‘Tora’ growing on medium with the addition of copper ions was significantly restricted in terms of the stomata.
3. The studied willow cultivar growing on medium with the highest copper salt dose was characterised by significantly higher values of the photosynthetic water use efficiency coefficient and intrinsic photosynthetic water use coefficient.
4. The addition of copper salt to the medium resulted in a decrease in RWC and an increase in WSD in the studied basket willow cultivar.

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