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Effect of iron deficiency and excess on biometric and biochemical parameters indicated in the radish sprouts (*Raphanus sativus* L. Subvar. *radicula* pers.)

Wpływ niedoboru i nadmiaru żelaza na parametry biometryczne i biochemiczne oznaczone w kiełkach rzodkiewki (*Raphanus sativus* L. Subvar. *radicula* pers.)

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Abstract
The aim of this study was to assess the changes of biometric and biochemical parameters in the radish sprouts (*Raphanus sativus* L. subvar. *radicula* Pers.), depending on the content of ions Fe^{III+} in the hydroponic culture. Seedlings were grown in Hoagland's complete hydroponic culture without Fe^{III+} ions or with the addition of FeIII+ ions in an amount of 30 and 300 mg/l. During the experiment, the plants were illuminated by a sodium vapor lamp Son-T-Agro-400 W Philips (40 μE•m²/s PAR at the medium level). After 7 days, some biometric and biochemical measurements were made: shoot and embryonic root length (cm), catalase (CAT) and peroxidase (POX) activity and antioxidant capacity. It was observed that Fe^{III+} added to hydroponic culture caused changes in the activity of enzymes and biometric parameters in radish sprouts (*Raphanus sativus* L. subvar. *radicula* Pers.).

Streszczenie
Celem badań była ocena zmiany parametrów biometrycznych i biochemicznych w kiełkach przeznaczonych do spożycia, rzodkiewki (*Raphanus sativus* L. subvar. *radicula* Pers.), w zależności od zawartości w podłożu jonów Fe^{III+}. Doświadczenie przeprowadzono w kulturach wodnych, wypełnionych pełną pożywką Hoaglanda. Kombinacje stanowiły rośliny rosnące w pożywce Hoaglanda bez jonów Fe^{III+}, pożywce zawierającej dodatek jonów Fe^{III+} w ilości 30 mg/l oraz 300 mg/l. W trakcie doświadczenia rośliny były oświetlane lampą sodową Son-T-Agro-400W, firmy Philips, o natężeniu promieniowania na poziomie 40 μE•m2•s^{-1} PAR. Po 7 dniach dokonano pomiarów biometrycznych: długość pędu (cm), długość korzeni zarodkowych (cm) oraz pomiarów biochemicznych: aktywność enzymów antyoksydacyjnych (CAT, POX) i pojemność antyoksydacyjną. Zastosowane w doświadczeniu jony Fe^{III+} wpływają na zmianę aktywności enzymów oraz parametry biometryczne w kiełkach rzodkiewki (*Raphanus sativus* L. subvar. *radicula* Pers.).

1. INTRODUCTION

In times of intense industrialization and economic development, the level of environmental pollution has dramatically increased. This situation forces us to monitor the concentration of metal ions and toxic substances in soil and air samples. Especially plants demand special form and high amount of nutrients in soil. Next to essential elements such as oxygen, hydrogen and carbon, plants need macro- and trace elements including heavy metals such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and molybdenum (Mo), required for the normal growth and development of plants. The concentration of iron in plants is considerably higher than the content of other microelements and (approximately 100–150 mg•kg^{-1} s.m.) [Starck 2002]. Iron is taken up by plants as Fe^{II+} and Fe^{III+} ions or as chelate compounds when the soil pH is low (3.5–6), while high content of calcium in soil makes iron unavailable to plants [Starck 2002; Kabata-Pendias, Pendias 1999]. Iron is a component of many important proteins and enzymes that regulate metabolism and plant growth [Agarwal et al. 2006]. Iron ions mainly regulate redox reactions [Kozlowska-Strawska 2010; Zarzecka 2004] and are cofactors of many enzymes such as superoxide dismutase (Fe-SOD), catalase, peroxidase and nitrate reductase. A large pool of the iron is present in porphyrins (cytochromes and ferredoxin) [Agarwal et al. 2006; Orlikowska, Zawadzka 2009]. Inadequate proportion of this element affects the formation of reactive oxygen species (ROS) in plants. Imbalance between ROS and antioxidant level in cells is referred as oxidative stress [Sheu et al. 2006]. Excess concentration of ROS affects destructively on all biomolecules, including proteins, lipids, carbohydrates and nucleotides damaging them and causing some chemical modifications [Bailey et al. 2005].

Many new scientific reports refer to healthful properties of plant seedlings. Plant seeds and seedlings contain a lot of valuable components such as vitamins, unsaturated fatty acids, enzymes, proteins, carbohydrates and macro- or trace elements, including iron. This is why sprouts are a valuable diet supplement and can protect organism against diseases of affluence like many types of cancers and cardiovascular disease. It is proved that water and ethanol sprout extracts have antioxidant properties and react with peroxides due to the substantial content of phenolic compounds and glycosides [Calzuola et al. 2004]. Antioxidant activity of sprouts depends on cultivation conditions [Yang et al. 2001]. The production of sprout is relatively easy and it is possible to modify their composition [Lewicki 2010].

2. MATERIAL AND METHODS

The aim of this study was to determine influence of iron ion (III) on biometric and biochemical parameters in radish sprouts (*Raphanus sativus* L. subvar. *radicula* Pers).

This study was conducted under controlled conditions in the laboratory of the Department of Biochemistry at the West Pomeranian University of Technology in Szczecin. Seedling were grown in Hoagland's complete hydroponic culture medium (pH 5.8), which contained 0.6 mg Fe^{III+}/l (control group), Hoagland's hydroponic culture medium without Fe III+ ions and with the addition of 30 mg Fe^{III+}/l or 300 mg Fe^{III+}/l. During the experiment, the plants were illuminated by a sodium lamp Son-T-Agro-400 W Philips, the intensity of radiation was 40 $\mu E \cdot m^2 \cdot s^{-1}$ PAR with an 8-hour light/8-hour dark photoperiod. The temperature was 18–20°C and the humidity about 75%.

After 7 days, biometric measurements like shoot length (cm), length of the embryonic root (cm) and biochemical measurements: the activity of antioxidant enzymes (CAT, POX) and antioxidant capacity were performed. Catalase activity measurement was made according to the method of Lück [1963], whereas peroxidase activity was determined by Chance and Maehly [1955] method. Antioxidant capacity was determined according to method described by Prieto et al. [1999]. There were three replications for each analysis. The results were analyzed statistically using one-way ANOVA and Tukey's HSD test at significance level $\alpha = 0.05$ (using the Statistica program 10.0).

3. RESULTS AND DISCUSSION

Plants can take up iron from the soil water in the form of salt ions Fe^{II+} or Fe^{III+}. Iron deficiency (limestone soil) or excess is common in the environment [Snowden, Wheeler 1993], and the plant absorption of Fe is dependent on the abundance of this element in the environment. Plants absorb substances from the soil selectively and in case of eventual excessive concentration of minerals in the environment, they adopt to new conditions [Strebeyko 1974]. This kind of connection is noticeable even within a one plant species, what was shown by the results of this experiment.

Limited plant growth caused by iron deficiency or toxicity was observed in many studies and described by various parameters, such as the accumulation of biomass, shoot and root growth, dimension and number of leaves, relative growth rate, etc. [Snowden, Wheeler 1993]. Strebeyko [1974] reported that iron deficiency results not only in a reduction of shoot and root growth but also in a decrease of leaf surface and biomass.

In the present study, it was found that the different concentrations of iron (III) had a visible effect on the plant organ growth (Table 1).

Table 1. Influence of iron ion Fe^{III+} on shoot and root length compared with the control group (cm)

Concentration of Fe^{III+} in medium	Shoot length (cm)	Root length (cm)
Control	4.055^{b}	2.687^{a}
I deficiency Fe^{III+}	3.108^{c}	1.764^{c}
II 30 mg Fe^{III+}/l	4.485^{a}	2.543^{b}
III 300 mg Fe^{III+}/l	2.383^{d}	1.100^{d}

Deficiency of iron in Hoagland's medium significantly reduced shoot and root length of radish sprouts (*Raphanus sativus* L. subvar. *radicula* Pers.) (Fig. 1). Iron added to Hoagland's medium in amount of 300 mg/l influenced a significant difference in the shoot growth and root length compared with control. The results showed that the highest concentrations of iron ion Fe^{III+} influence significantly on shoot and root length, inhibiting it more than 41% or 59%, respectively, compared with the control group. However, it is said that toxic symptoms of iron are not characteristic, and that the growth of plant is not limited by this metal [Kabata-Pendias, Pendias 1999]. In this study, the best conditions for the growth of radish were observed in a medium with 30 mg Fe^{III+}/l.

Heavy metals, despite some morphological changes, cause metabolic disorders. Iron is one of the transition elements, which participate in the formation of RFT in oxidation and reduction reactions. It takes part in the formation of $OH^{\bullet}$ and H_2O_2 in Fenton reaction and initiate lipid peroxidation [Rucińska-Sobkowiak 2010]. Caro and Puntarulo [1996] noticed that excess of iron leads to increased concentration of hydroxyl radical and singlet oxygen in soybean roots. The appearance of ROS in plant cells activates a number of defense mechanisms, for example, activity of catalase and peroxidase, the enzymes that detoxify harmful hydrogen peroxide. These studies showed influence of iron ions onto enzymes activity in studied plant, which was possibly associated with higher production of hydrogen peroxide in terms of stress (Fig. 1).

Both deficiency and excess of Fe^{III+} in medium have significant influence on enzyme activity in radish sprouts (*Raphanus sativus* L. subvar. *radicula* Pers). Peroxidase activity in plant treated with Fe increased significantly compared with control. In plants grown in medium with the deficiency of Fe^{III+}, the activity of enzymes increased by 33.94%, whereas the enzyme activity in plants collected from the medium with an excess of Fe (30 mg/l) increased by 109.43%. The most serious stimulation of enzyme activity was observed in plants growing in the medium with 300 mg Fe^{III+}/l (peroxidase activity was three times higher than in control group – up to 350%). Pooyan et al. [2008] observed an increase in peroxidase activity in rice, whereas the activity of catalase decreased (compared with control plants).

However, in our study, it was observed that the deficiency of iron (III) in culture medium significantly increased the activity of catalase (CAT) up to 157.4% of control (Fig. 2). Iron ions added to medium culture caused not serious increase in enzyme activity (30 mg Fe^{III+}/l – by 4.4% and 300 mg Fe^{III+}/l – by about 5.5%). A similar relation was observed by Buenos and Piqueras [2002] in tobacco.

Antioxidant capacity ORAC (oxygen radical absorbance capacity) determines the superoxide binding, both in terms of size and time of the binding [Cao, Prior 1999]. In our study, significant differences in this parameter were observed. The highest oxygen radical absorbance capacity was noticed for plants growing in medium supplemented with 300 mg Fe^{III+}/l (Fig. 3).

4. SUMMARY

1. Iron doses applied in the experiment influenced significantly on some biometric and biochemical parameters in radish sprouts (*Raphanus sativus* L. subvar. *radicula* Pers.).
2. Doses of Fe^{III+} used in the experiment affected the activity of catalase and peroxidase. A significant increase in the peroxidase activity was observed, both for deficiency and excess of iron (III) in the culture medium.
3. The highest ORAC was observed in plants grown in culture medium supplemented with 300 mg Fe^{III+}/l.

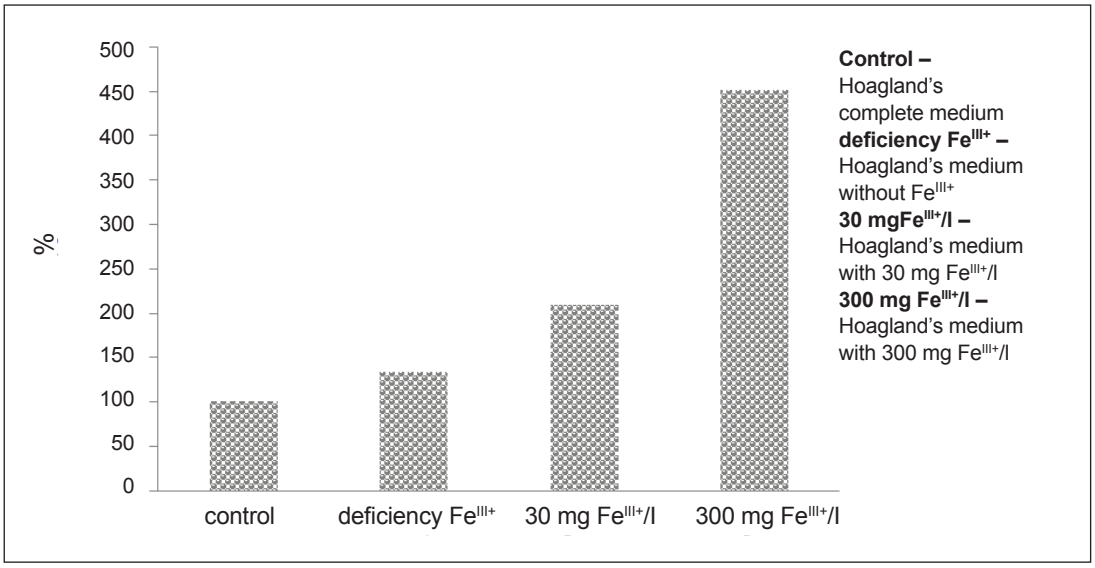

Fig. 1. Influence of iron ion Fe^{III+} on peroxidase activity in radish sprouts compared with the control group (%).

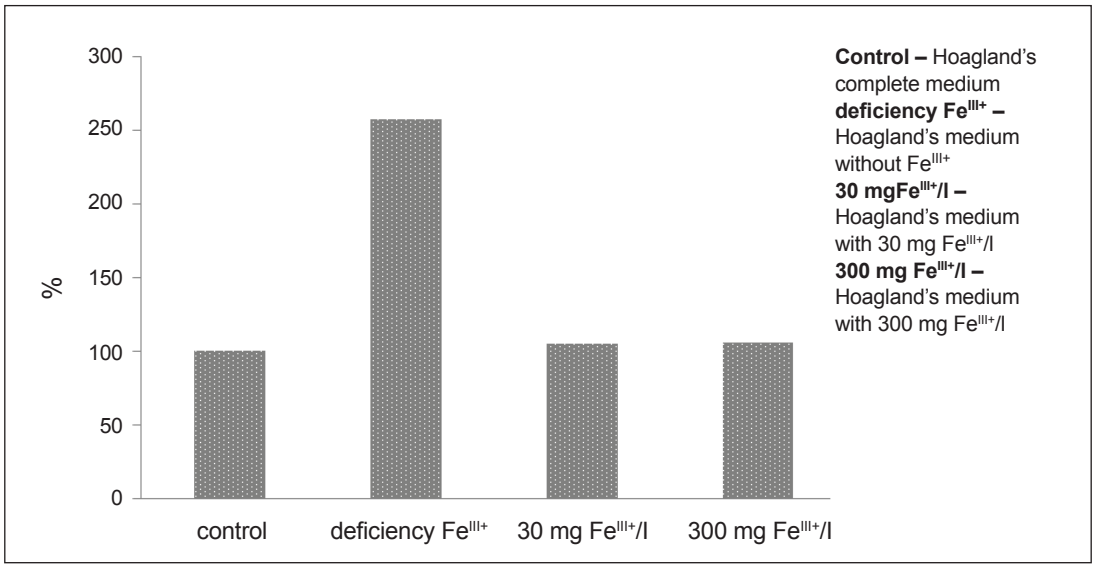

Fig. 2. Influence of iron ion Fe^{III+} on catalase activity in radish sprouts compared with the control group (%).

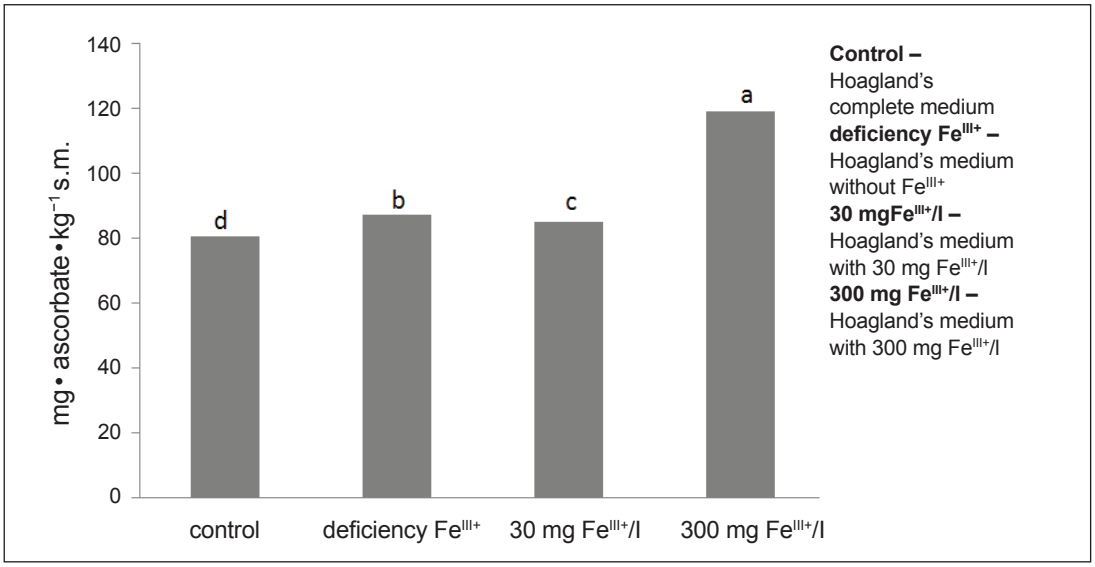

Fig. 3. Influence of iron ion Fe^{III+} on the antioxidant capacity in radish sprouts compared with the control group.

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