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Effect of yttrium on photosynthesis and water relations in young maize plants

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Abstract: Despite an increase in spectrum of industrial applications of yttrium (Y) and the fact that it is widely present in the soils and plants, some of which are agronomically important crops, its effects on plant growth and metabolism are still obscure. Therefore, the aim of this work was to examine the effect of different concentrations of Y on its accumulation and distribution, photosynthetic responses, water relations, free proline concentration and growth of young maize plants. The experiment was done with maize (*Zea mays* L., hybrid NS-640), in water cultures, under semi-controlled conditions of a greenhouse. Plants were supplied with half-strength complete Hoagland nutrient solution, to which was added either 0 (control), 10^{-5}, 10^{-4} or 10^{-3} mol/L Y, in the form of $Y(NO)_3{\cdot}5H_2O$. Each variant was set in thirteen replications, with six plants in each replication. Plants were grown for 21 d and they were at the stage of 3 and 4 leaves when they were analyzed. The presence of Y reduced maize growth and photosynthetic performance. Dimensions of stomata significantly decreased while their density significantly increased on both adaxial and abaxial epidermis. Plant height, root length, total leaf area and dry mass also declined. Concentration of photosynthetic pigments (chl *a* and *b* and carotenoids) and free proline decreased. Photosynthesis and transpiration were impaired in the presence of Y – their intensities were also reduced, and the same stands for stomatal conductance of water vapor, photosynthetic water use efficiency (WUE) and water content. Although the highest concentration of Y was found in maize roots in each treatment, Y concentration in the second leaf and shoot also significantly increased with an increase in Y concentration in the nutrient solution. Albeit Y concentration was much higher in roots than in shoots, shoot metabolism and growth were much more disrupted. The results demonstrated that young maize plants accumulated significant amount of Y and that this element, when present in higher concentrations, had unfavorable effect on physiological processes and therefore plant growth.

Keywords: yttrium; maize; photosynthesis; water use efficiency; rare earths

Yttrium (Y) and the other rare earth elements (REEs), including 15 lanthanides and lanthanum, possess nearly identical chemical and physical properties and comprise a homogenous group of elements in the periodic system. To the contrary of their name, they are not rare but rather widely distributed (present) in the nature[1]. Concentration of particular REEs in the biosphere is at the level of the other microelements[2]. It was calculated[3] that the Earth's crust contains 24 mg/kg of $_{39}$Y, which is about three times less than zinc or copper but about 2.5 times more than lead. The lack of techniques that are sufficiently sensitive for the quantitative assessment of their presence in the atmosphere and limited interest in studies about toxicity aspects of REEs are two main reasons for their insufficient research in the past. There has been a growing interest in the study of the REEs in recent decades of the last century, with the exploitation of REEs resources and the applications in modern industry, agriculture, medicine and biotechnology. The main application of REEs as new materials for recent technologies in the modern industry are in the areas of fluorescent and superconducting materials, permanent magnet, magnetic bible memory, solid state laser[4,5]. In agriculture, low concentrations of REEs-based fertilizers are used to increase yield and quality of crops[6]. Essentiality of REEs for living organisms or their threat to the environment has not been identified so far. There are no precise evaluations for the long-term biological effect of REEs on the plants and other living organisms. Although knowledge on RREs is limited, it seems unlikely that REEs pollution may cause any large-scale severe environmental problems in the near future[7].

Beneficial, toxic or nil effects of REEs on plant metabolism, growth and crop yield were observed in controlled and field conditions[8,9] which are similar to those of other trace metals. Little fundamental information about the influence of individual REEs on metabolism and growth of vascular plants is available. Using mixtures of REEs makes it difficult to conclude whether any single element is of particular importance. Of all REEs, the largest number of papers was published on lanthanum (La) and cerium (Ce), especially compared with Y,

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probably because their concentration in plants is 10–100 times higher than concentration of other REEs[10].

The first study on the uptake of Y in plants was done in the fifties of the last century. The mankind was then for the first time confronted with the danger of entering of radioactive fission products in the food chain[11,12]. In nuclear fission reactions twenty six unstable Y isotopes have been characterized[13]. Because of this, the study of the uptake of Y in plants gained importance, in particular after Chernobyl disaster in 1986[14], because in nuclear fission reactions various isotopes of Y are produced in rather large amounts. It was found that ferns and lichens accumulate higher amounts of Y as compared to other genera[15].

Thus far, the essentiality of REEs for higher plants was not identified. Nevertheless, there are a number of results demonstrating their positive effect on the growth and yield of plants[16]. It is possible that in the future some of the REEs will be also recognized as essential or beneficial mineral elements for plants or just at least for some plant species[17]. There are numerous data indicating that Y influences activity of certain enzymes, but not directly physiological processes in plants[18]. In our earlier work we showed that different concentrations of Y have significant effect on growth of sunflower[19]. In this regard, the goal of this study was to investigate the effect of Y on photosynthesis and water regime as well as physiological and morphological characteristics of maize, an agronomically important crop, on which these processes depend.

1 Experimental

1.1 Plant material and growth conditions

Maize (*Zea mays* L. hybrid NS-640) seeds were surface sterilized with 70% ethyl alcohol and 5% hydrogen peroxide and germinated in the dark at 25 ºC, on sterilized quartz sand and watered daily with dematerialized water. Uniform 6-days seedlings were transferred on half-strength Hoagland solution[20] to which was added either 0 (control), 10^{-5}, 10^{-4} or 10^{-3} mol/L Y. Yttrium was added as $Y(NO)_3{\cdot}5H_2O$ (Merck). Each variant was set in thirteen replications, with six plants in each replication. The plants were grown in the greenhouse under irradiation of 200–300 μmol quanta/m^2/s, day/night temperature of 24±2/15±2 ºC and relative humidity of 65%–75%. Nutrient solution was changed every day and plants were aerated regularly. Plants were grown for 21 d and they were in the stage of 3 and 4 leaves when they were harvested. Shoots, roots, and second leaves of each plant were separated before the analyses.

1.2 Growth analysis

Fresh matter of shoots, roots and second leaves was measured and dry matter was recorded after drying the samples at 70 ºC to constant mass. Total leaf area was measured by an automatic area meter (LI-300, Li-Cor, Lincoln, USA). Height of the shoot and length of root of each plant was measured by ruler.

1.3 Concentration of chloroplast pigments

Concentrations of chlorophyll *a* and *b* and carotenoids were determined spectrophotometrically, in the acetone extract of freshly harvested second leaves, using molar extinction coefficients of[21,22].

1.4 Photosynthesis and transpiration

Photosynthesis, transpiration, stomatal conductance and substomatal cavity CO_2 concentration were measured using LC pro+Portable Photosynthesis System, manufactured by ADC BioScientific Ltd. Light conditions were set using the LCpro+ light unit, which emitted photosynthetically active radiation (PAR) at 1000 μmol/m^2/s. The air supply unit provided a flow of ambient air to the leaf chamber at a constant rate of 100 μmol/s. Humidity was set at 10 mBar of partial water pressure. Temperature and CO_2 concentration were at ambient levels. Measurement was conducted in 3 replication on 3 plants per one treatment (9 measurments per treatment in total). Parameter WUE (water use efficiency) was calculated as the ratio of photosynthesis to transpiration and expressed in μmoles of CO_2/m^2/s/mmol of H_2O m^2/s. Stomatal conductance of water vapor (g_s) and substomatal cavity CO_2 concentration (*C*i) were measured automatically and expressed in units mol H_2O m^2/s for g_s and μmol/mol for *C*i.

1.5 Concentration of free proline

Proline concentration in roots and shoots was determined according to the method described in Ref. [23]. Approximately 1 g of plant material was homogenized in 10 mL 3% aqueus sulfosalicyclic acid and filtered through Whatman's filter paper. Two mililitres of filtrate were mixed with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at temperature of 100 °C. The reaction mixture was extracted with 4 mL toluene, and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Beckman, USA Duferies 60 spectrometer. Appropriate proline standards were included for calculation of proline concentration in the sample in three replications.

1.6 Density and size of stomata

Stomatal density and size were determined on the second leaf. Light microscopy observations of leaf adaxial and abaxial epidermal prints were done following the procedure described in Ref. [24]. Stomatal density was determined by counting stomata in 25 microscope fields