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The effect of lanthanides on the accumulation of rubber (1,4-Cis-polyizoprene) in Dandelion Crimean-Sagyz (*Taraxacum Hybernum*) Under adverse climate, and its environmental consequences

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ABSTRACT
The stimulating effect of lanthanides on the accumulation of rubber in the roots of dandelion *Taraxacum hybernum* (Krym-saghyz) was demonstrated along with its environmental consequences. The treatment of Krym-saghyz plants with nitrates of lanthanides led to the increase of poly (cis-1.4-isoprene) concentration by 80% compared to the untreated plants and by 45% compared to those treated with magnesium nitrate. The observed increase of rubber concentration in Krym-saghyz plants has been assumed to be related to the activation of photosynthesis (up to 40%) and the inclusion in the reactions of rubber synthesis of isopentenyl pyrophosphate (IPP) formed from methylerythritol phosphate (MEP) in green plastids.

Keywords: *Taraxacum hybernum*, Rubber crop, NMR-spectroscopy, Poly (cis-1.4-isoprene), Lanthanides, CO_2-assimilation intensity, Environmental issues.

INTRODUCTION
Natural rubber (NR) is the foremost important natural resource after air, water and petrol (Cornish 2014). It is produced from latex *Hevea brasiliensis* Muell Arg, mainly in Southeast of Asia, where the fungus, *Microcyclus ulei* Henn., lethal for rubber trees has not been registered yet (Rocha *et al*. 2011). Obviously, the contamination of the *H. brasiliensis* plantations by this fungus in the Southeast Asia and Africa is a matter of time, that is why a particular attention has been given to a search for the alternate natural rubber crops (van Beilen & Poirier 2007; Cornish 2017). The Russian dandelion (*Taraxacum kok-saghyz* Rodin) is often considered as an alternative that produces the rubber similar to the *H. brasiliensis* one (Cornish *et al.* 2012; Hosseini et al. 2018). That is why there are many experimental works dedicated to *T. kok-saghyz*. The genetic diversity of this species was studied (Arias *et al.* 2016; McAssey *et al.* 2016). The transcriptomic analysis of *T. kok-saghyz* was carried out (*Luo et al.* 2017; Kavyanifar *et al.* 2020). To boost yield productivity of *T. kok-saghyz*, the investigations on the optimization of inorganic nutrition are carried out and the approaches for the industrial growing of this species are working out (Eggert *et al*. 2018). In the first half of the 20th century, two dandelion species, *T. kok-saghyz* (Lipshitz 1934) and *T. hybernum* (Dogatkin 1947), were cultivated in the Soviet Union for obtaining of the NR. *T. hybernum* produces less rubber but at the same time has higher biomass compared to *T. kok-saghyz. T. kok-saghyz* roots contain 10-14% of rubber in the dry weight and have a productivity of 20-50 centners of fresh

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weight per hectare, and a maximum of productivity never exceeds 70 centners per hectare (Kazakevich 1942). *T. hybernum* contains 4-7% of rubber in the dry weight of roots, and its productivity is 160-180 centners of root fresh weight per hectare, at the maximum of 330 centners per hectare (Bondarenko 1941). Besides, the root system of *T. hybernum* is less branched, and the life cycle implies leaf fall in the period of summer heat, which makes the crop harvesting easier. Consequently, the dandelion *T. hybernum* offers a variety of advantages that promote to use it as a raw material for rubber production. Krym-saghyz was cultivated on the man-made irrigated fields in Middle Asia's republics of the USSR. It has been considered that due to the productivity of its root mass, product yield from 1 hectare, as well as to its technical characteristics, *T. hybernum* is promising for commercial production (Bondarenko 1941). *T. hybernum* seeds, unlike the *T. kok-saghyz* ones, were not delivered to the Soviet Union's allies during the World War II. Because of this or for any other reasons, there were no publications over the last 60 years, which discussed the potentials of *T. hybernum* to be used as an alternative rubber source. If the *H. brasiliensis* plantations would be infected by fungus *M. ulei*, the production of NR from alternative sources would be extremely necessary within the shortest time span, foremost, from the rubber-containing roots of dandelions. The genetic modification of a reliable plant line of dandelions can take years. Chemical stimulation of rubber production is preferable. It is known that the usage of a stimulating paste with ethephon on the *H. brasiliensis* plantations increases the production of latex by the plants (Derouet *et al.* 2003). To increase the *H. brasiliensis* productivity it is also advised to control the levels of Mg^{2+} in the soil (da Costa *et al.* 2006). Consequently, the theoretical prerequisites for increasing the productivity of rubber crops with the help of chemical treatment do exist. Lanthanide elements (Ln^{+3}) can be used as such chemicals. It is known that Ln^{+3} (La^{+3}, Ce^{+3}, Nd^{+3}, Gd^{+3}, Eu^{+3}) at low dosage can stimulate growth and photosynthesis of microalgae (Goecke *et al*. 2015), exert favourable effects on assimilation intensity of CO_2 and productivity of barley (Rico *et al*. 2015), increase photosynthetic and antioxidant levels in spinach plants (Chao *et al*. 2009; Yin *et al*. 2009; Ze *et al*. 2009), and restore the chlorophyll biosynthesis as well as carbonic and nitrogen metabolism in corn (Zhou *et al*. 2011; Zhao *et al*. 2012). Lanthanide elements are considered to substitute the effects of bivalent ions (Ca^{2+}, Mg^{2+}). However, their action is unlikely to be restricted only to the substitution of Ca^{2+}, Mg^{2+}. Novel investigations were performed where the possibility of Ln-mediated modulation of signaling pathways that led to the mobilization of physiological processes in plants was discussed (Hagenbeek *et al.* 2000; Li *et al.* 2016; Liu *et al*. 2016). It might be assumed that the use of Ln^{+3} as the stimulators of plant physiological processes could have a positive effect on the synthesis of rubber in dandelion. NR in plants is synthesized with the help of cis-prenyltransferase (CPT) located in membranes of rubber particles. Biosynthesis of rubber depends on the concentration of allylic pyrophosphate (APP), which serves as the initiator of the polymerization reaction, isopentenyl pyrophosphate (IPP), which is a rubber monomer, and Mg^{2+} ions necessary to activate the polymerization reaction (Cornish, 2014). For CPTs, Mg^{2+} is not only a co-factor (Scott *et al*. 2003; da Costa *et al.* 2005) but also a regulator of their activity (da Costa *et al.* 2006). The chief purpose of the present study was the examination of lanthanides stimulate rubber biosynthesis under moderate climate as well as its environmental consequences where the rubber productivity of Krym-saghyz dandelion is low.

MATERIALS AND METHODS

Plant material

Seeds of Krym-saghyz dandelion (*T. hybernum* Steven) collected in December 2015 around Nikita Botanical Garden (Crimea) were planted in 0.5 l containers with a commercial soil «Terra vita» in February 2017. Thinning was carried out after the emergence of seedlings, leaving 3 plants in each container. Cultivation was carried out in growth chamber under 22°C with photoperiod 16/8 and photoactive radiation intensity of 150 $\mu mol\ m^{-2}\ s^{-1}$. Sprouts were spray treated with 0.01 molar solution of lanthanum nitrate ($La(NO_3)_3 \times 5H_2O$), ytterbium ($Yb(NO_3)_3 \times 5H_2O$), magnesium ($Mg(NO_3)_2$) and potassium (KNO_3) (for control) in April (performed in four biological replicates). At the beginning of May, the plants were planted into the open soil on the territory of Kazan Federal University. *T. hybernum* plants at the generative ontogenetic stage (q_2) collected around Sevastopol at the late September 2017 were taken as a reference sample.

Quantitative determination of technical product by gravimetric analysis

The extraction of the technical product (TP, rubber + resin) from 3 g of pulverized lyophilized roots was carried out in 80 ml methylbenzene (Post *et al*. 2012). The extracted TP was dried and weighted.

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Determination of poly(cis-1,4-isoprene) by ^{1}H-NMR-spectroscopy

0.03 g of TP was dissolved in 760 µL of deuterated chloroform-d$_3$ ($CDCl_3$ Sigma-Aldrich) and analyzed by ^{1}H-NMR. All NMR spectra were obtained at 30°C on a Bruker Avance III 600 MHz NMR spectrometer equipped with an inverse 5 mm TXI Probe. The ^{1}H-NMR spectra were recorded using a one-dimensional ^{1}H-NMR pulse program with a 30°-pulse. For each sample 90-degree pulse calibration procedure has been done.

Only one scan was acquired for ^{1}H-NMR spectra to reduce error of integration of NMR signals arising due to relaxation processes. The residual proton signal of the deuterated chloroform-d$_3$ (7.24 ppm) was used for spectra calibration. All NMR data were processed and analyzed using Topspin 3.5 software. The amount of poly(cis-1,4-isoprene) in the deuterated chloroform solution was determined by comparing the peak intensities of the C_5 methyl protons (Schmidt *et al.* 2010) with the corresponding signals for a poly(cis-1,4-isoprene) (Post *et al.* 2012). The concentration of poly (cis-1,4-isoprene) in the extracts was determined relative to the extract of the reference sample (*T. hybernum* grown under natural conditions in Crimea).

Determination of CO_2 assimilation intensity

Photosynthesis intensity (CO_2 assimilation intensity - A) was registered by gas-measuring mobile system GFS – 3000 (Heinz Walz GmbH, Germany) under natural illumination, humidity and CO_2 concentration. Measuring was carried out at the end of September – beginning of October. Photoactive radiation intensity was 119 ± 39 µmol m^{-2} s^{-1}, CO_2 concentration was 419 ± 2 ppm, and humidity of 45 ± 0.7%. Average values of CO_2 assimilation intensity were calculated relative to the area of the leaves.

Statistical analysis of the results

All experiments were performed in 4 biological replicates. Statistical analysis of the results was carried out by OriginPro 9.0 Program. Authenticity of differences between the variants was estimated by Mann Whitney U test ($P < 0.05$).

RESULTS AND DISCUSSION

The average concentration of TP in the dry weight of the wild plant roots *T. hybernum* after 2 years of vegetation does not exceed 6,6 ± 1.4% of the air-dry weight according to Lapin (Lapin 1936). In roots of *T. hybernum* used in our experiments as a reference sample, the output of TP was 5,5 ± 1,1% (Table 1).

Table 1. The content of the technical product (TP) and poly (cis-1,4-isoprene) in the roots of *Taraxacum hybernum* grown in the presence of KNO_3 (control), $Mg(NO_3)$, $La(NO_3)_3$, $Yb(NO_3)_3$, on the territory of Kazan Federal University as well as grown under natural conditions in Crimea (reference sample). The stars point to the significance of differences between the variants treated with Mg $(NO_3)_2$ and treated with lanthanide elements (n = 3). The significance was estimated by Mann Whitney U test ($P < 0.05$).

Sample	Reference	Control (KNO_3)	$Mg(NO_3)_2$	$La(NO_3)_3$	$Yb(NO_3)_3$
Content of TP in the dry weight of roots (%)	5.5 ± 1.1	2.0 ± 0.5	3.3 ± 0.45	2.9 ± 0.7	2.6 ± 0.7
Content of poly(cis-1,4-isoprene), in % compared to the reference sample	100	3.6 ± 0.9	10.6 ± 1.3	17.2 ± 1.5*	18.7 ± 2.4*

After the first year of vegetation under natural conditions of Middle Asia, the yield of TP was 3-5% (Bondarenko 1941). In our experiments, the content of TP did not exceed 3.3 ± 0.45% (Table 1). Since TP consists not only of rubber but also of a resin, the quality of TP was estimated by the amount of poly (cis-1,4-isoprene) using ^{1}H-NMR-spectroscopy. The highest peaks of poly (cis-1,4-isoprene) were observed for the extracts of the reference sample (Fig. 1). The samples of plants treated with potassium nitrate, magnesium nitrate, and lanthanide elements contained 3.6%, 10%, and 17-18%, respectively, of poly (cis-1,4-isoprene) compared to the reference sample (Table 1). The differences can be explained by the following.

Introduction of exogenous Mg^{2+} as an additional co-factor for both the reactions of IPP synthesis from mevalonic acid (MVA) in the cytosol and polymerization reactions in rubber particles, where magnesium regulates the

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activity of CPT (Scott *et al*. 2003; da Costa *et al*. 2005; da Costa *et al.* 2006; Cornish 2014) led to an increase in poly(cis-1,4-isoprene) concentration in the roots of *T. hybernum*.

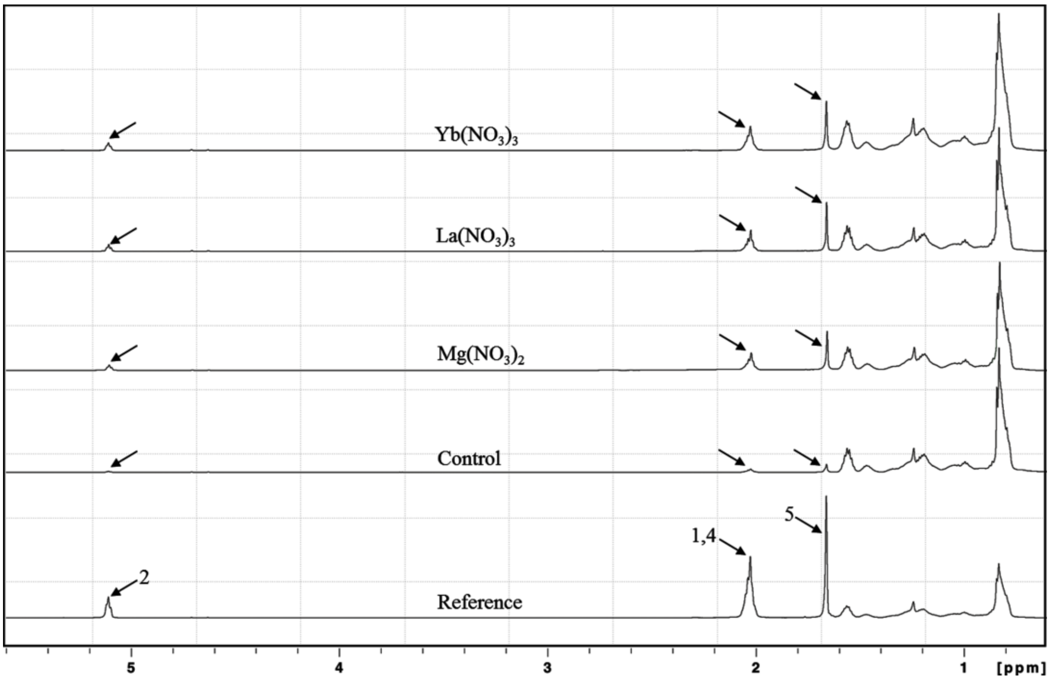

Fig. 1. [1]H-NMR spectra of root extracts from *Taraxacum hybernum* plants grown in the presence of KNO_3 (control), $Mg(NO_3)$, $La(NO_3)_3$, $Yb(NO_3)_3$, on the territory of Kazan Federal University as well as grown under natural conditions in Crimea (reference sample). The arrows indicate residual signals of the cis-configured polymer. The numbers refer to proton signals that are related to the corresponding carbon atoms in poly(cis-1,4-isoprene).

Lanthanide elements in addition to their role as magnesium agonists might also have a positive influence on the level of photosynthesis. Earlier, such kind of effect of lanthanide elements was demonstrated (Chao *et al.* 2009; Yin *et al.* 2009; Ze *et al*. 2009). Photosynthesis intensification, in turn, can lead to the increase of cytosolic concentration of IPP synthesized from MEP in green plastids. It is known that IPP synthesized from MEP may diffuse from the plastids and join the cytosolic pool (Cornish 2014). In turn, at high concentration, IPP is used for the synthesis of poly (cis-1,4-isoprene). To test it, we measured the photosynthesis intensity in *T. hybernum* untreated or treated with magnesium or lanthanide elements. Fig. 2 demonstrates the results of measurements of CO_2 assimilation intensity in *T. hybernum.* Under low photoactive radiation (around 100 µmol m^{-2} s^{-1}), the plants treated with lanthanide elements assimilated CO_2 by 40% more than the control and treated with Mg^{2+} plants. At the same time, when lanthanide elements were applied, the content of poly (cis-1,4-isoprene) was increased compared to the control group by 80% and compared to the group treated with magnesium by 45% (Table 1).

SUMMARY

It is commonly considered that IPP synthesized by MVA-pathway serves as a substrate for CPTs localized on the rubber particles in *H. brasiliensis* during the synthesis of poly (cis-1,4-isoprene) (Sando *et al.* 2008). At the same time, *HbDXR2* gene of MEP pathway *H. brasiliensis* encodes one of the functional enzymes that takes part in the synthesis of the NR (Ko *et al.* 2003). So, it is considered that IPP synthesized both by MEP and MVA pathways may simultaneously be utilized by CPTs in the course of the production of NR (Ko *et al.* 2003).

CONCLUSION

Thus, we have shown that the treatment of the juvenile plants of *T. hybernum* with lanthanum and ytterbium nitrates increases the CO_2 assimilation intensity as well as the concentration of poly (cis-1,4-isoprene). The latter is likely to be due to the utilization by CPTs of IPP synthesized by MEP pathway. Therefore, lanthanide elements may be applied for increasing a rubber production by dandelion plants.

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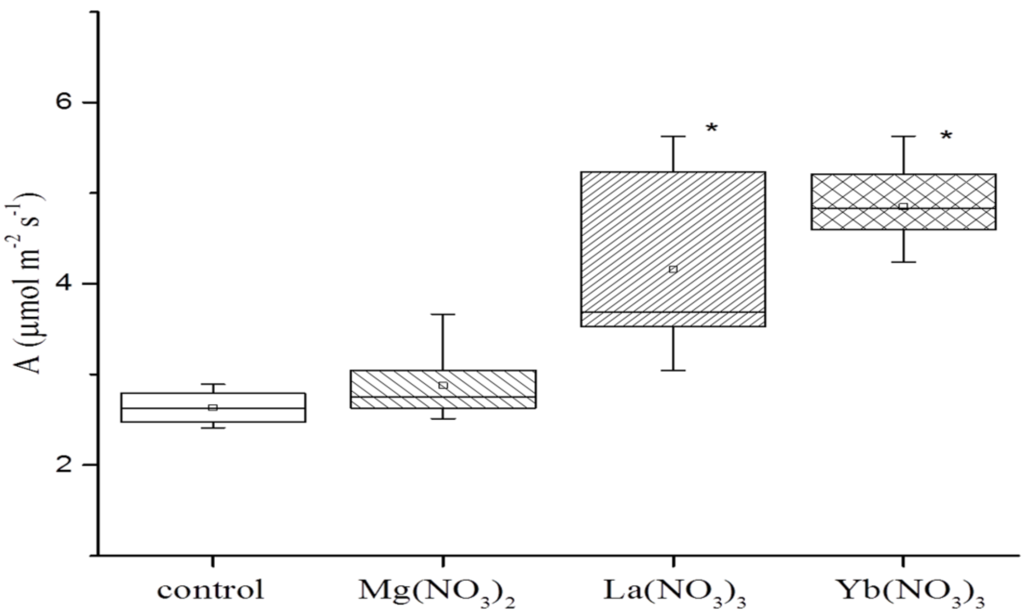

Fig. 2. Photosynthesis intensity (CO_2 assimilation intensity - A) in *Taraxacum hybernum* plants untreated (control) or treated with $Mg(NO_3)_2$, $La(NO_3)_3$, $Yb(NO_3)_3$. The stars point to the significance of differences between the variants treated with $Mg(NO_3)_2$ and treated with lanthanide elements (n=9). The significance was estimated by Mann Whitney U test ($P < 0.05$).

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اثر لانتانیدها بر تجمع رزین (Cis-polyizoprene-۱،۴) در قاصدک کریمه-ساگیز (*Taraxacum hybernum*) تحت آب و هوای نامساعد، و پیامدهای زیست محیطی آن

والدمیر نیکولاویچ وروب[۱]، بولات ایماموت دینویچ خیروالدینو[۲]، سرگی فدرویچ کتو[۳]، ورا والدمیرنوا نیکولنکو[۴]، الکساندر لئودینویچ میخایلو[۵]

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چکیده

در مطالعه حاضر، اثر محرک لانتانیدها بر تجمع رزین در ریشه قاصدک Taraxacum hybernum (Krym-saghyz) همراه با عواقب زیست محیطی آن نشان داده شد. تیمار گیاهان کریم-ساغیز با نیترات‌های لانتانید منجر به افزایش غلظت پلی (سیس -۴-ایزوپرن) ۸۰ درصد در مقایسه با گیاهان درمان نشده و ۴۵ ٪ در مقایسه با گیاهان تیمار شده با نیترات منیزیم شد. فرض بر این است که افزایش مشاهده شده غلظت رزین در گیاهان Krym-saghyz مربوط به فعال سازی فتوسنتز (تا ۴۰٪) و گنجاندن در واکنش‌های سنتز رزینی ایزوپنتنیل پیرو فسفات (IPP) تشکیل شده از متیل اریتریتول فسفات (MEP) در پلاستیدهای سبز است.

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