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SHORT COMMUNICATION

BIOACCUMULATION AND PHYSIOLOGICAL IMPACT OF RARE EARTH ELEMENTS ON WHEAT (*TRITICUM AESTIVUM*)

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Rare earth elements (REEs) frequently occur together in rare earth minerals and have similarities in ionic radii and chemical activities with other elements in periodic table. REEs have beneficial effects on plant growth and soil properties. The aim of this study was to evaluate the influence of REEs on plant (Wheat) growth and their accumulation of rare earth elements. Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) was carried out to assess the fractionation of REEs in different tissues of wheat plant. The mean concentrations of rare earth elements were determined in roots, shoots and leaves of wheat. A greater biomass and significant accumulation of REEs were inferred in roots than in leaves and shoots. Increased levels of Chlorophyll, amylase and Superoxide dismutase (SOD) activity were observed in the REEs treated plants. This study implicates the role of REEs as fertilizers to improve plant biomass production.

Key words: **Bioaccumulation, rare earth elements, wheat**

The rare earths are a group of 17 elements composed of 15 Lanthanides, Scandium and Yttrium. The Lanthanides denote a group of 15 elements with atomic numbers between 57 and 71. The Indian Rare Earth Limited (IRE) has processing plants at Manavalakurichi, Kanyakumari district, Tamil Nadu, Chavara, Kollam district, Kerala and Orissa Sand Complex OSCOM, Matikhalo, Ganjan district, Orissa. All plants contain naturally occurring lanthanides, also commonly known as REEs. The occurrence of lanthanides in plants was first reported in 1880 (Cossa, 1880). Lanthanide concentration ranging from few nanograms (ng) to few micrograms (μg) per gram of dry plant material (wheat and corn) in plants varies among different plant species with the growth environment and among different plant tissues (Zhang *et al.*, 2001). Fractionation of rare earth takes place in plants depending on the radius or the redox potential. A small concentration of rare earth elements in the form of soluble ions exist either on the cell walls of apoplasts or in the intercellular spaces or on cell membranes. The transfer of metal ions to the aerial parts is mainly through xylem (Liang *et al.*, 2008).

Rare earth rich soil in China is being used as manure in chinese agriculture. (Guo, 1986), (Brown *et al.*, 1990). Though the study of REEs toxic effects are in progress, no detailed study has been reported on the relationship between rare earth elements and plants in India. In the present study, bioaccumulation and influence of REEs on wheat plant has been investigated. REEs rich soil samples were collected from Manavalakurichi (Kanyakumari District, Tamil Nadu, South India). The samples were collected in sterile polythene cover and brought to the laboratory for analysis. The known weight

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of collected soil samples was acid digested with 3:1 ratio of HCl and HNO_3 respectively, and volume was made up to 10 ml with distilled water (Hill *et al.*, 2002). The digested soil samples were analyzed for the evaluation of REEs concentration using Inductively Coupled Plasma-Mass Spectrometry (Perkin Elmer Sciex ELAN DRC II.ICP-MS).

Wheat seeds (*Triticum aestivum var. UP270*) were treated with 1% sodium hypochlorite and rinsed clean of any traces of sodium hypochlorite. They were soaked in 0.2, 0.4 and 0.6 % soil solution rich in REE for 24 hours. The treated seeds were placed in sterile petri plates containing cotton soaked with sterile water to provide moist condition for sprouting. Then they were allowed to grow on pots containing garden soil (sterilized).

Growth of wheat plants as affected by REE treatment was measured in terms of length and dry weights of root and shoot. The presence of REEs in different parts of wheat plant i.e., leaf, root and shoot was measured following acid digestion (Wang *et al.*, 2001) with use of Inductively Coupled Plasma Optical Emission Spectrometry(Optima 5300 DV ICP- OES).

Amylase enzyme was extracted from sprouted seeds with 0.1 N phosphate buffer (pH 6.7). The enzyme assay was carried out by DNS method (Palanivelu, 2001). Assay of SOD was based on the inhibition of formation of NADPH-phenazine methosulfate nitro blue tetrazolium formazon. The SOD activity of leaves was assayed spectrophotometrically (Kakkar *et al.*, 1984). Chlorophyll was extracted with 80% acetone and the absorption at 663 nm and 645 nm were read in spectrophotometer (Witham *et al.*, 1971).

Table 1. Rare Earth Element Concentration in Soil of Manavalakurichi as measured on ICP-MS.

S. No.	Analyte	Type of REE	Mass	Conc. Mean (ppm)
1.	Pr	Light	141	55.88
2.	Yb	Heavy	172	23.72
3.	Eu	Heavy	151	0.33
4.	Ce	Light	140	478.22
5.	Nd	Light	146	201.62
6.	Sm	Light	147	43.11
7.	Gd	Heavy	157	40.81
8.	Tb	Heavy	159	0.00
9.	Dy	Heavy	163	41.72
10.	Er	Heavy	166	20.37
11.	Tm	Heavy	169	4.54
12.	Ho	Heavy	165	10.80

The soil sample collected from Manavalakurichi (Kanyakumari District,Tamil Nadu, India) showed an abundance of Cerium and Neodymium (Table 1 ; 478.22 and 201.62 ppm respectively). An increase in root and shoot length (mean value: 7.83 and 8.68 respectively), dry weight of root and shoot (mean value: 0.06 and 0.05 respectively) was observed with REEs compared to non REE treatment (Table 2). An insight into tissue REE level revealed maximum accumulation of Cerium and Neodymium in roots followed by shoots and leaf tissues. A higher input of REEs i.e. at 0.6 % yields high accumulation when compared to lower inputs 0.2 % and 0.4 % (Table 3). We observed a higher level of seed amylase, antioxidant (SOD) activity and an increased level of chlorophyll content in leaves of REE treated than the untreated plants.

Table 2. Plant biomass production as affected by REEs.

S.No	Treatment	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Root length (cm)	Shoot length (cm)
1.	With REE	0.05 ± 0.01	0.06 ± 0.01	7.83 ± 0.54	8.68 ± 0.25
2.	Without REE	0.02 ± 0.06	0.05 ± 0.01	3.96 ± 0.74	6.24 ± 0.20

Values are mean ± SD of triplicates.
Statistically significant ($P<0.05$) compared to untreated seeds

Table 3. Accumulation of REEs in plant tissues.

S. No.	Sample Composition		REEs accumulation (ppm)	
			Cerium	Neodymium
1	0.2 % Soil sample	root	1.04	0.04
2	0.4 % Soil sample	root	5.01	0.5
3	0.6 % Soil sample	root	7.08	1.3
4	0.2 % Soil sample	shoot	0.91	0.84
5	0.4 % Soil sample	shoot	2.1	1.87
6	0.6 % Soil sample	shoot	4.9	3.09
7	0.2 % Soil sample	leaf	0.4	0.92
8	0.4 % Soil sample	leaf	1.1	1.36
9	0.6 % Soil sample	leaf	2.7	2.04

BDL – Below detectable limit (Ce- < 0.0480 mg/L and Nd- < 0.0960 mg/L)

Parthasarathy *et al.* (1986) studied about the lanthanide contents of Manavalakurichi monazite and reported a variation 55.6 to 46.8 % in the Light Rare Earth elements (LREEs) including Ce and Nd, while the Heavy Rare Earth elements (HREEs) vary from 1.55 to 0.908 %. In the present study, a similar type of distribution of LREEs especially Ce and Nd were found to be rich in the soil samples of Manavalakurichi. The communication by Jeya *et al.* (2008) also reported the presence of Ce and Nd in monazite sample which was comparatively higher than other REEs in the samples of both Chavara and Manavalakurichi. Hence in the present study, Ce and Nd were considered for their accumulation and impact on growth of plants Wheat. Jarvan in 2002 observed that the soaking of seeds in REEs solutions at different time intervals increased the mass of young plants, wheat kernels after 24-hour-soaking with REE solutions increased the plant mass by 20.4-26.2%.

Table 4. Seed Amylase ,Leaf SOD and Chlorophyll level of wheat seedling as affected by REE.

Parameters	With REE	Without REE
Amylase*	3.14 ± 0.240	2.72 ± 0.132
SOD (U/gm)	14.97 ± 0.261	13.92± 0.123
Total Chlorophyll (mg/gm)	1.14 ± 0.021	1.07 ± 0.015

*Enzyme Activity is expressed in units;
1 unit = 1 nmol Maltose utilized/ mg of protein /min.
U – unit = Activity of enzymes that gives 50% inhibition of the extent of NBT reduction in 1 min.
The results are expressed as the mean values of six experiments ± SD
Statistically significant (P<0.05) with respect to the controls

In the present study, greater biomass and increased root growth were observed after the exposure to different lanthanide concentrations. Roots have higher REE concentrations than the other plant organs such as stems and leaves. REE concentrations are elevated in roots and they decrease in the order of roots > shoot > leaves (Wyttenbach *et al.*, 1998). The bioavailability of nutrients and ions depend largely on the physico-chemical and biological characteristics of soils, especially the dynamic processes in the rhizosphere (Zhang and Cao, 1999).

Rare earth elements could enhance chlorophyll content and improve photosynthetic rate, and thus, could increase plant biomass. Spraying rare earth on pepper foliars (He *et al.*, 1998) improved the total chlorophyll content. Many studies suggest that REEs can stimulate plants to absorb, transfer and assimilate nutrients. Significant levels of Ce and Nd occurred in different parts of wheat plant such as root, shoot and leaves respectively. LREEs such as Ce and Nd enrichment were observed in soil sample. Higher levels of REEs accumulation were noticed in seeds treated with 0.6% concentration of REEs. It can be concluded that REEs have a profound effect on the chlorophyll content and SOD level. Increased activity of enzymes like amylase in the presence of REEs may influence the coleoptile formation and biomass production.

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