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Efficacy of rare earth elements on the physiological and biochemical characteristics of *Zea mays* L.

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Abstract

In the present study, bioaccumulation of REEs in *Zea mays* L. (corn plant) has been analyzed. The role of Rare Earth Element (REEs) in physiology and biochemistry of corn plant have been studied. Physiological characters like peroxidase (POD) and superoxide dismutase (SOD) activities, biochemical analysis of chlorophyll, carotenoids and phenol contents have also been carried out. The plant growth and biomass observed shows the influence of REEs and the accumulation studies reveal the presence of REEs only in roots. It was also found that REEs could increase POD and SOD activities, biochemical content and had an impact on crop production and stress resistance in plants.

Keywords: Rare earth elements, bioaccumulation, anti oxidative enzymes, ICP-MS

Introduction

Rare earth elements are applied to improve crop production, and the distribution patterns of individual rare earth elements in native plants have widely been reported. But the knowledge is still limited about the dose-dependent accumulation of individual rare earth elements in agricultural crops after application of rare earth elements (Xu, 2003). Application of mixtures of rare earth elements at >10 mg kg^{-1} soil, resulted in significant increase in contents of light rare earth elements in the roots, and at a dose of 50 mg kg^{-1} soil, a similar phenomenon was found at the plant tops. Rare earth elements (REEs) frequently occur together as rare earth minerals and have similarities in ionic radii and chemical activities (Henderson, 1984). Although they are called rare earth elements because of their similarity to the earth (i.e., magnesia, lime, etc.), REEs are not all rare but represent a group of 15th most abundant component of the earth's crust. The activity or certain plant enzymes have been found to be enhanced by the application of REE (Brown et al., 1990). Application of REEs in agriculture has been carried out intensively since 1972, aiming at increasing crop yields. Chinese researchers have reported both physiological and yield responses with no adverse environmental effects (Xiong, 1995). With this regard, many research works have been done to show the beneficial effects of REEs on plant growth and soil properties which stimulate the synthesis of chlorophyll (Guo, 1988), to promote seedling development (Chang, 1991; Wu et al., 1983), to stimulate root and shoot growth in crops such as (*Triticum aestivum* L.), cucumber (*Cucumis sativus* L.), soybean [*Glycine max* (L.) Merr.], and corn using both pot and plot experiments. REEs in plants showed dose-dependent accumulation (Wu et al., 1983). Effects of REEs on POD activity of tea plant were also analyzed by Wang et al. (2003). Much less work has been done on the adverse effects of REEs. Many studies have reported the accumulation of RE in different types of cereal crops or in different parts of plants (Liu et al., 1997; Lao et al., 1996). There is an increasing interest in the bioaccumulation processes of REEs due to the wide application of REEs in a variety of non-nuclear industries and agriculture, resulting in possible environmental contamination (Choppin et al., 1986; Wang et al., 2001). Meanwhile, due to their unique chemical structures, they may be used to trace the sources of inorganic elements in plants (Fu et al., 2001; Fu and Tasuku, 2000). Investigations into the bioaccumulation characteristics of REEs have been carried out in recent years as sensitive techniques such as inductively coupled plasma-mass spectrometry has become available (Fu et al., 2001; Wei et al., 2001). Concentrations of REEs in plants are extremely variable, with about 700 ng g^{-1} of La has been reported in a new species of fern (Matteuccia) (Fu et al., 1998), but it can be less than 10 ng g^{-1} La in the needles of Norway spruce (Wyttenbach et al., 1994). In India, much less work has been concentrated upon the bioaccumulation and effects of REEs on corn plants. Hence in the present study, fractionation of REEs in various parts of corn plant was analyzed and the effect of REEs on biochemical and physiol-

Table 1. ICP-MS for soil sample

S. No.	REE analyzed	Atomic Mass	Measured intensity mean	Concentration of the element Mean (ppm)
1	Praseodymium (Pr)	140.9077	228925.74	55.88
2	Ytterbium (Yb)	173.04	20947.70	23.72
3	Europium (Eu)	151.964	715.62	0.33
4	Lanthanum (La)	138.9055	776252.22	239.23
5	Cerium (Ce)	140.116	151325.38	201.60
6	Samarium (Sm)	150.36	26046.86	43.11
7	Gadolinium (Gd)	157.25	32570.64	40.81
8	Terbium (Tb)	158.9253	29760.64	0.00
9	Dysprosium (Dy)	162.5	42883.70	41.72
10	Erbium (Er)	167.259	33585.92	20.36
11	Thulium (Tm)	168.9342	14324.07	4.54
12	Neodymium (Nd)	144.24	34027.16	10.80

ogical characteristics of corn plant have been carried out.

Materials and methods

Sample collection

REEs rich soil samples were collected in a sterile polythene cover from Manavalakurichi and brought to the laboratory for analysis.

Assessment of REEs in the soil

Known weight of collected soil samples were acid digested with 3:1 ratio of HCL and HNO_3 respectively. It was made up to 10 ml with distilled water. The digested soil samples were analyzed for the evaluation of REEs concentration using ICP-MS (Perkin Elmer Sciex ELAN DRC II).

Seed treatment and sprouting

Before sprouting, the corn seeds were washed with 1 % sodium hypochlorite. Then they were washed with distilled water to remove the excess of Sodium hypochlorite solution. The seeds were then soaked in 0.2, 0.4 and 0.6 % of REE solution of different concentration for about 24 hours. They were placed in a required moisture condition for sprouting. The sprouted seeds were grown on sterile soil till a measuring level of their growth. Spraying of mineral solution (Hoagland's solution) was done at regular intervals on plants while hardening.

Fractionation and accumulation of REEs

The presence of REEs was measured in diverse parts of corn plant (leaf, root and shoot). Fractionation of plants was done and acid digestion was carried out. Accumulation in the plant samples were analyzed using ICP-OES (Optima 5300 DV ICP- OES).

Estimation of POD in leaves

In the presence of a hydrogen donor like pyrogallol or dianisidine, peroxidase converts H_2O_2 into water and oxygen. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be measured using a spectrophotometer at 430 nm. A known amount of leaves (0.5 g) were homogenized in 2.5 ml of phosphate buffer (0.1 M). Then the mixture was subjected to centrifugation, and the supernatant was used as the enzyme source. 0.2 ml of the enzyme extract along with 3 ml of pyrogallol solution (0.05 M in 0.1 M phosphate buffer pH 6.5) was taken in a cuvette. 0.5 ml of H_2O_2 was added to the reaction mixture and the change in absorbance was recorded at every 3 minutes. One unit of peroxidase activity is defined as the change in absorbance per minute at 430 nm (Reddy et al., 1985).

Estimation of SOD in leaves

Assay of SOD is based on the inhibition of formation of NADPH-phenazine methosulfate nitro blue tetrazolium formazon. The residual chromogen can be extracted using an organic solvent like butanol. The colour formed at the end of the reaction can be measured at 630 nm using a spectrophotometer, as a measure of SOD activity. The leaves (0.5 g) were homogenized with 3ml of potassium phosphate buffer (0.025 M pH 8.3) and the suspension was centrifuged at 5000 rpm for 10 minutes. 0.2ml of the supernatant was then mixed with 1.2 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 ml of phenazine methosulfate(186 μM),0.3 ml of Nitro blue tetrazolium (300 μM) and 0.2 ml of NaOH. The reaction mixture was incubated at 30°C for 90 seconds. Then the reaction was stopped by adding 1ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4 ml of butanol and allowed to stand for 10 minutes. It was then centrifuged and the intensity of the chromogen in the butanol layer was measured at 560 nm against butanol as blank. The reaction mixture devoid of the enzyme source served as control. One unit of enzyme activity is defined as the enzyme reaction that gave 50 % inhibition of NBT reduction in one minute (Kakkar et al., 1984).

Estimation of phenols in leaves

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to produce blue coloured complex quantified at 650 nm. 0.5 gm of leaves were homo-

Table 2. Accumulation of REEs in plant tissues

S. No.	Sample ID	Sample Composition	REEs accumulation (mg/L)	
			La	Ce
1	AR	0.2 % element 1 + root	1.04	0.96
2	BR	0.4 % element 1+ root	5.01	4.82
3	CR	0.6 % element 1+ root	7.08	6.95
4	AS	0.2 % element 1+ shoot	0.91	0.92
5	BS	0.4 % element 1+ shoot	2.10	2.22
6	CS	0.6 % element 1+ shoot	4.90	5.24
7	AL	0.2 % element 1+ leaf	0.45	0.59
8	BL	0.4 % element 1+ root	1.10	1.82
9	CL	0.6 % element 1+ leaf	2.79	2.55

A – 0.2%, B – 0.4% and C – 0.6%
L – Leaf, S – Shoot and R – Root

genized in 10X volume of 80 % ethanol, and centrifuged at 10000 rpm for 20 minutes. The residue was reextracted with 80 % ethanol, the supernatant was evaporated to dryness and dissolved in distilled water with known volume (0.5 ml) of Folin-Ciocalteau reagent. After 3 minutes 2 ml of 20 % sodium carbonate was added and the mixture was placed in a boiling waterbath for 1 minute. The amount of phenol in the extract was then quantified using a spectrophotometer at 650 nm. A standard catechol solution corresponding to 2- 10 µg concentration was added to Folin-Ciocalteau reagent and sodium carbonate. A standard curve was constructed using an electronic calculator on the linear regression mode using which, the concentration of phenol in samples was read. The values were expressed as milligrams of phenol per gram of leaf (Mallik and Singh, 1980).

Estimation of chlorophyll in leaves

Chlorophyll was extracted with 80 % acetone and the absorption at 663 nm and 645 nm were read in spectrophotometer. Using the absorption co-efficient the amount of chlorophyll was calculated. One gram of leaf was extracted with 20 ml of 80 % acetone and centrifuged at 5000 rpm for 5 minutes. The supernatant was transferred to a fresh flask until the residue was colourless. The supernatant was made up to 100 ml with 80 % acetone. The absorbance of the solution was read at 645 nm and 663 nm against 80 % acetone blank and the amount of chlorophyll was estimated (Witham et al., 1971).

Estimation of Carotenoids

Total carotenoids can be extracted in petroleum ether and estimated in UV-visible spectrophotometer at 450 nm. 0.5 gm of leaf was homogenized and saponified for about 30 minutes in a shaking water bath at 37°C with a specific volume of 12 % alcoholic KOH. The saponified extract was transferred into a separating funnel containing 10-15 ml of petroleum ether (40-60°C) and mixed well. The lower aqueous phase was transferred to another separating funnel and the upper petroleum ether containing carotenoid pigment was collected separately. The extraction was repeated until the aqueous phase was colourless. A small quantity of anhydrous sodium sulphate was added to the petroleum ether extract, to remove the turbidity. Absorbance of the extract was measured at 450 nm using a spectrophotometer with petroleum ether as blank (Zakaria et al., 1979).

Assessment of biomass

The physical parameters are the direct measurements of the corn biomass (with and without treatment of REEs). The overall biomass of corn plant was evaluated using parameters like length of leaves, root and shoot, wet weight and dry weight of root and shoot. The obtained data were statistically analyzed using Agres Statistical Software Version 3.01 (1994).

Results and discussion

The soil samples collected from Manavalakurichi were analyzed. ICP-MS showed the distribution of rare earth elements in the samples (Table 1). Although at low concentrations, contents of individual rare earths in overgr-ound plant samples were similar between control and treated group ranking in the order of Ce > La > Nd > Pr > Sm; another concentration order of La > Ce > Nd > Pr > Sm was noticed when rare earths were applied at higher doses, clearly indicating the incorporation of exogenous rare earths into plant tissues (Wang et al., 2001). The concentrations of REEs in unpolished rice from Kočani Field exhibited a similarly elevated HREE pattern, like paddy soils, with up to 6.6 x 10^3 times lower values compared to those in the soil. The accumulation coefficients (La - Sm) indicated no preferential fractionation of La-Sm in the rice-paddy soil system of Kočani Field (Nastja et al., 2006). Lanthanum and Cerium were abundant in the analyzed samples in the present study (239.23 and 201.60 ppm respectively in Soil sample). The digested diverse parts of plant samples (root, shoot and leaves) were analyzed for REE concentrations in the samples with ICP-OES (Table 2). It revealed maximum accumulation of REEs (Lanthanum and Cerium) in plant tissues. A higher input of REEs (0.6 %) rendered high accumulation when compared to lower inputs (0.2 % and 0.4 %). Plant accumulation of metals from soils depend both on the availability of the metal and on physiological processes. Soil factors such as cations and pH influence the proportion of a soil metal which is bioavailable. The ionic form of the metal is usually considered to be the bioavailable form, although ligand-enhanced uptake of Cd has been demonstrated (Berkelaar and Hale, 2003a and b). Plant show no preference on individual RE and the results of fingerprinting indicated clearly the incorporation of exogenous REs in plant tissues, in a similar manner as that observed in the dose-dependent distribution of RE concentrations. The results also indicated a translocation process of REs from plant root to leaf when applied to soil or from leaf to root when applied to leaf. A homeostatic regulation mechanism for excessive uptake of REEs in plants was suggested to regulate the concentrations of REs in the plant (Wang et al., 2001). The path for REE transportation from soil to plant top can be divided into four parts: soil, rhizosphere, root and xylem vessel. Chemical reactions in soil and rhizosphere affect REE fractionations before uptake by roots. After the transfer of REEs into roots,

Table 3. Physiological and Biochemical parameters of corn leaves in REEs treated and untreated samples

Parameters	With Element	Without Element
POD (U*/gm)	85.22 ± 1.025	65.41 ± 0.732
SOD (U^+/gm)	14.97 ± 0.261	13.92 ± 0.123
Total Phenols(mg/gm)	15.25 ± 0.302	14.01 ± 0.53
Total Chlorophyll (mg/gm)	1.35 ± 0.025	1.15 ± 0.018
Total carotenoids(mg/gm)	9.35 ± 0.059	8.59 ± 0.082

U* - unit = change in absorbance / min at 430 nm

U^+ - unit = Activity of enzymes that gives 50% inhibition of the extent of NBT reduction in 1 min.

Values are mean ± SD of triplicates.

Statistically significant ($P<0.05$) compared to untreated seeds

REE fractionations were controlled by fixation mechanisms when entering the xylem vessels. Then they were transferred to the aerial parts of the plant (Ding et al., 2007). Antioxidant enzymes like POD and SOD activity on corn leaves along with estimated chlorophyll, carotenoids and phenol content is given in Table 3. The physiological responses include more chlorophyll, enhanced rate of development, greater production of roots, stronger tillering, etc. The reported yield responses include increased dry and fresh matter in crop plants such as wheat, increased vitamin C in plants. The concentration of La in plant tissues generally increased both in leaves and roots as a result of La application. This perhaps influenced the increase in yield without any detrimental effect of the uptake of both macro- and micro-nutrients, as exhibited in plant tissue analyses. The physical parameters of the corn biomass were evaluated (root and shoot length, wet and dry weight of root and shoot) with and without REEs. Table 4 shows that a significant increase in root and shoot length (mean value: 7.51 and 5.83 respectively), dry weight of root and shoot (mean value: 0.03 and 0.03 respectively) and wet weight of root and shoot (mean value: 0.09 and 0.08 respectively) treated with REEs compared to other trials. The effect of La (III) complex on germination, coleoptiles, and root length of two local varieties of wheat for different treatment periods has been investigated and the complex was found to exhibit enhanced activity (Gudasi et al., 2006). The different REE patterns among different parts of individual plants reflect the different mobility of REEs in plants. The REE patterns are steeper for leaf and stem than that for secondary root and at the same time parts with steeper patterns usually show smaller amounts of REEs (Fu et al., 2001). Only a few countries have documented crop responses to application of REEs. The significance of REEs for agricultural plant production, begin with a description of the history of their application. The recorded physiological and biochemical effects and responses of select crops to REEs were reviewed by Hu et al., (2004). Crop responses to REE application were reported by the Chinese researchers to be most probable when soils contain less than 10 mg kg^{-1} of available rare earths. Rare earth elements increased the ability of resistance to drought by maize. It also enhances the activity of plant hormones, seed germination etc. In plants the accumulation of rare earth element is higher in roots than other parts of plants. Many studies suggest that REEs can stimulate plants to absorb, transfer and assimilate nutrients. Ning and Xiao (1989) reported that after using REEs as fertilizers, the absorption of rice for N, P, and K is increased by 16.4 % 12 %, and 8.5 %, respectively. 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 decreased in the order roots > shoot > leaves (Wyttenbach et al., 1998). Distribution and localization of REEs in plants and plant organs, describe the soil–plant relationship and interactions. It also shows the adverse effects on plant growth, crop production and their importance in plant physiology and biochemistry (Tyler, 2004). Seed treatment and spraying with REE during the growth has increased the kernel yield in vessel trials by 5.2 % - 14 %, depending on the soil (Xie and Chang, 1985). In the field trials carried out by the same scientist in different parts of China, in which the seed was treated and plants sprayed with REE solutions, the yield of spring wheat increased on an average of four years by 11 %. The yield increase was due to a bigger number of kernels in the head and a bigger 1000- kernel mass. This increase in above yield indicators as well as in the number of productive shoots due to REE was also been observed by other researchers (Shi et al., 1994). The distribution of REE among the main organs of vascular plants differs consider- ably. However, roots have usually higher concentrations than other plant organs and this is only partly due to the fact that it might be difficult to liberate soil–growing roots from soil particles. Roots of maize and mungbean grown in solution culture accumulated 20–150 times higher La concentrations than their shoots (Diatloff et al., 1995). Solutions containing REEs sprayed on crops may be translocated in the plant tissues, and there are even indications that movements of REEs may take place from leaf to root, as studied in maize (Wang et al., 2001). Generally the uptake rate of REEs from soil to root was much higher than the translocation rate from root to shoot (Hu et al., 2004) . Physiologists indicate that REEs can increase the activities of photosystem-II in plant and bind to its chlorophyll. It has also been reported that La^{3+} accelerated the photosynthetic reactions at suitable concentration *in vivo* (Hong et al., 2001). When mixtures of rare earth elements were replaced by lanthanum alone, at a dose higher than 10 mg La kg^{-1} soil, a significant increase in La content occurred in the roots and in the plant tops. The content ratio of La to Ce in maize plants appeared to increase as the application doses of rare earth element(s) increased. At the highest dose (50 mg kg^{-1}soil), the transport of the absorbed La from the roots to the plant tops might be substantially reduced after treatment with lanthanum alone, compared with mixtures of rare earth elements (Xu et al., 2003). Rare earth micronutrient fertilizer (REMF) could promote POD activity in tea shoots and the tea shoot biomass was enhanced. Wang et al., (2003) reported that the POD protein in tea shoots was found to bind rare earth elements, mainly La, Nd and Ce. Possible reasons for the differences in REE concentrations in plants include differences in REE concentrations in soils and differences among plant species (Miekeley et al., 1994; Fu et al., 2001). Like other trace elements, REE concentrations were elevated in roots and they decreased in the order of

Table 4. Influence of REEs on growth and biomass of corn plant

S.no.	Samples	With Element						Without Element					
		Length of		Wet weight		Dry Weight		Length		Wet weight		Dry Weight	
		Root (cm)	Shoot (cm)	Root (g)	Shoot (g)	Root (g)	Shoot (g)	Root (cm)	Shoot (cm)	Root (g)	Shoot (g)	Root (g)	Shoot (g)
1	I	8.3	5.9	0.08	0.07	0.02	0.01	6.1	3.2	0.1	0.13	0.06	0.07
2	II	6.3	6.6	0.09	0.08	0.03	0.01	5.3	2.5	0.07	0.05	0.05	0.04
3	III	8.8	3.2	0.1	0.12	0.02	0.04	5.5	4.2	0.07	0.06	0.04	0.05
4	IV	9.3	5.9	0.13	0.1	0.05	0.03	6.3	4.5	0.09	0.07	0.03	0.01
5	V	5.8	7.3	0.07	0.06	0.02	0.01	7.2	6.2	0.05	0.04	0.02	0.01
6	VI	8.7	7.1	0.06	0.09	0.04	0.03	6.5	3.4	0.09	0.15	0.09	0.04
7	VII	5.9	6.4	0.1	0.06	0.02	0.01	4.7	2.4	0.09	0.07	0.04	0.09
8	VIII	8.1	3.8	0.09	0.10	0.01	0.05	5.1	4.0	0.06	0.04	0.06	0.06
9	IX	9.1	5.2	0.15	0.1	0.07	0.04	6.4	4.7	0.1	0.05	0.05	0.03
10	X	4.8	6.9	0.04	0.05	0.01	0.03	6.7	6.4	0.07	0.02	0.01	0.01
	Mean	7.51	5.83	0.09	0.08	0.03	0.03	5.98	4.15	0.08	0.07	0.05	0.04

roots > leaves > seeds (Wyttenbach et al., 1998). However, the reasons for this kind of variation are not clear and more investigation on the accumulation of REEs in plants are needed. It may be concluded that the REEs have a profound effect on the cultivation of corn by improving crop production, enzymatic activities and stress resistance.

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