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A Study on Impact of Rare Earth Elements and Bacteria in Growth of Wheat (*Triticum aestivum*)

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Abstract

The present study was investigated to study the impact of Rare earth elements in growth of wheat. The soil samples were collected from rare earth environments and analyzed by ICP-MS to know constituents of the rare earth elements. Cerium and Neodymium are abundant in the analyzed samples. The physical parameters of the wheat biomass are evaluated with and without treatment of REEs and also treated with REEs and a bacterial strain (*Pseudomonas* sp.,). From the results it is evident that a significant increase in root and shoot length, dry weight and wet weight of plants treated with REEs. The production stress resistant end product (phenol) is measured and the total content of soluble phenols is calculated. Seeds treated with element alone show two fold increases in the production of phenol. The diverse parts of plant samples (root, shoot and leaves) are digested analyzed by ICP-OES analysis. It reveals that a maximum accumulation of REEs (Cerium and Neodymium) in plant tissues and higher input of REEs yields high accumulation and lower inputs yield low accumulation. The seeds treated with culture are shown drastic change in the accumulation of REEs by wheat.

1. Introduction

The term rare earth arises from the minerals from which they were isolated, which were uncommon oxide type minerals (earth). Rare earth elements are named in honor of the scientist who discovered the elemental properties. There is a large amount of reserves of rare earth elements in the world. The largest proved reserves are located in China. Other important reserves are located in Australia, Russia, United States, Brazil, Canada and India. In addition some deposits of rare earth were found in South Africa, Sri Lanka, Indonesia, Malaysia, etc. The rare earth reserves of India are estimated to be 2.7 million tons. The principal sources of rare earth are the minerals bastnasite, monazite, loparite and the lateritic ion adsorption clays.

The accumulation of rare earth takes place in plants also. The concentrations of rare earth elements in plants under natural conditions are extremely low for accurate determination. The types and concentrations of organic ligands in the rhizosphere may be the main factors causing the distribution patterns and levels of fractionation of rare earth elements in various plants. The accumulation of rare earth elements in the aerial parts can be reduced by the precipitation with phosphate. Concentration of rare earth elements in plants are extremely variable, with about 70 ng g^{-1} La reported in one species of fern (Fu *et al.*, 1998), less than 10 ng g^{-1} La in needles of Norway spruce (Wyttenbach *et al.*, 1994). The accumulation of rare earth element varies greatly under different pH values. Time dependent accumulation of rare earth in plants after their agricultural application also takes place.

Rare earth elements in plants induce metabolism as a result they are used as fertilizer in agriculture. Different species of plants have different sensitivity to rare earth elements ant their concentrations, and are also affected by the application time. The acute toxicity of rare earth using as a fertilizer is low. Fertilization with rare earth elements is economically more efficient, there is also an effect of synergism- the treatment with rare earth element and growth regulator together in the stage of shooting resulted in a bigger yield. Metabolism of nutrients in plants increased by rare earth elements by the transfer of N from inorganic to organic form, this is a benefit for protein synthesis and regulation of nutrient balance. Rare earth element in plants enhances the rate of respiration, increases the photosynthesis rate and decreases the loss of water. Spraying rare earth on pepper foliars improved the total chlorophyll content of chlorophyll-a, chlorophyll-b (He *et al.*, 2009). Rare earth increased the ability of resistance of drought by maize. It also enhance 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.

Kalagouda *et al.*, in 2006 studied the effect of complex on germination, coleoptiles and root length of wheat and comparative effect of complex, ligand, salt, methanol and revealed that the germination percentage of water treated seeds and complex treated seeds were not significantly different, germination was not affected by the treatments. In case of root length and shoot length the complex treated seeds showed increase in the length than the water treated ones.

Shi Ming *et al.*, in 2007 examined the bioaccumulation of extraneous REEs in field grown wheat during the growth cycle and investigated the fractionation of REEs during their transport in the soil-wheat system and observed that the accumulation of REE in wheat parts ranked in order of roots > leaves > stems > grains at maturity stage. The distribution pattern of REEs varied considerably at different stages. The present study was aimed to study the impact of Rare earth elements and the bacteria *Pseudomonas* sp. in the growth of Wheat.

2. Materials and Methods

Sample collection

Rare Earth Elements rich soil sample was collected from Chavara. The samples were collected in sterile polythene cover and brought to the laboratory for analysis.

Assessment of REEs in the soil

The known weight of collected soil samples were digested with 3:1 ratio of HCl and HNO_3 respectively. It was made up to 10 ml with distilled water. They were analyzed for the evaluation of Rare Earth Elements concentration using ICP-MS.

Seed treatment and sprouting

Before sprouting, the wheat seeds were washed with 1% Sodium hypochlorite and after that, they were washed with distilled water to remove the excess of Sodium chloride solution. They were soaked in Rare Earth Elements solution of different concentration for 2 hours. Another set of seeds were treated with Rare Earth Elements solution along with the bacterial strain. They were placed in a required moisture condition for sprouting.

Hardening

The sprouted seeds were let to grow in sterile soil till the growth achieves to a measuring level. It was important that spraying of mineral solution (Hoagland Solution) while hardening.

Assessment of biomass

The physical parameters are direct measurement of the wheat biomass were evaluated (root and shoot length, wet and dry weight of root and shoot) with and without treatment of Rare Earth Elements and also treated with Rare Earth Elements and a bacterial strain (*Pseudomonas sp.,* MTCC, IMTECs)

Measurement of phenol products

The method used for measurement of phenol was based upon Swain and Hillis, (1959) and the reaction according to Lam and Street, (1977) with slight modification. The samples to be measured for phenol were homogenized in 20ml of methanol 80% and agitated for 15 min at 70 °C. 200 µl of the methanolic extract were combined with 7ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1N) and the solution was kept at 25 °C. After 3 min at 25 °C, 1 ml of saturated solution of Na_2CO_3 and 1 ml of water were added and the reaction mixture was incubated for 1 hour at 250ºC. The absorption of the developed blue color was measured spectrophotometrically at 725 nm. The content of total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reaction with a phenol solution (C_6H_6O) of 1% and expressed as phenol equivalents in mg/g.

Fractionation and Accumulation of REEs using ICP -OES analysis

The diverse parts of plant samples (root, shoot and leaves) were digested with the mixture of 3:1 HCl: HNO_3 in a 50 ml beaker. The digested solution was evaporated to near dryness. The residue was dissolved by diluted HCl and made upto 10 ml with deionized water. Rare Earth Elements concentrations in the samples were determined by ICP-OES.

3. Results and Discussion

Rare earth soils are precious, but very common minerals across Arabian coast, especially in Chavara. Some studies show that the presence of rare earth soil, microorganism produces more organic compound, especially carboxylic acids than in their absence (Gu *et al.*, 2001). Chinese studies show that rare earth soil has a significant effect on the growth of plants. In India there is no study has been carried out on the relationship between rare earth elements and bacteria. Since Illuminate, Rutile, Zirconium is semi conducting constituents in soils in rare earth soils. The study on bioaccumulation of REEs is needed to investigate the adoptive behavior of plants. In the present study, bioaccumulation and fractionation of REEs by wheat have been investigated. The soil sample was collected from rare earth environments in coastal region of Chavara. They are analyzed by ICP-MS (PerkinElmer Sciex ELAN DRC II) to know constituents of the rare earth elements which was given in Table:1. Cerium and Neodymium are abundant in the analyzed and the concentration mean of the sample was 478.219 and 201.618 ppm respectively.

Table: 1 - ICP-MS Analysis of elements

S. No.	Analyte	Mass	Meas.intens mean	Conc. Mean (ppm)
1.	Pr	141	228925.744	55.885
2.	Yb	172	20947.707	23.726
3.	Eu	151	715.628	0.330
4.	Ce	140	1565768.364	478.219
5.	Nd	146	151345.399	201.618
6.	Sm	147	26046.861	43.117
7.	Gd	157	32570.642	40.810
8.	Tb	159	29760.642	0.003
9.	Dy	163	42883.708	41.723
10.	Er	166	33585.926	20.368
11.	Tm	169	14324.076	4.543
12.	Ho	165	34027.163	10.803

Table: 2- Measurement of wheat biomass with Rare earth elements

		With Element (Rare earth elements + seeds)						Without Element (Seeds alone)					
S.No	Samples	Length of		Wet weight of		Dry Weight of		Length of		Wet weight of		Dry Weight of	
		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	6.5	7.1	0.08	0.04	0.07	0.02	2.5	6	0.03	0.03	0.04	0.01
2	II	7.2	7.7	0.09	0.03	0.04	0.01	3.6	6.5	0.05	0.03	0.07	0.01
3	III	11.1	5.0	0.06	0.05	0.05	0.03	4.0	4.2	0.04	0.04	0.04	0.01
4	IV	7.5	5.7	0.07	0.05	0.06	0.01	4.1	5.3	0.06	0.05	0.06	0.01
5	V	7.5	9.0	0.08	0.05	0.07	0.02	4.5	6.3	0.03	0.02	0.05	0.01
6	VI	8.5	11.3	0.1	0.04	0.04	0.02	4.2	6.5	0.1	0.06	0.05	0.03
7	VII	7.1	11.0	0.12	0.1	0.04	0.03	4.8	7.2	0.13	0.05	0.06	0.04
8	VIII	10.1	10.8	0.09	0.09	0.07	0.05	3.5	6.8	0.06	0.07	0.04	0.02
9	IX	6.4	9.7	0.09	0.11	0.05	0.02	3.2	7.5	0.12	0.07	0.07	0.03
10	X	6.4	9.5	0.10	0.07	0.02	0.02	5.2	6.1	0.13	0.05	0.02	0.04
Mean		7.83	8.68	0.11	0.09	0.06	0.05	3.96	6.24	0.075	0.047	0.05	0.021

The physical parameters of the wheat biomass are evaluated (root and shoot length, wet and dry weight of root and shoot) with and without treatment of REEs and also treated with REEs and a bacterial strain (*Pseudomonas* sp.,). The results are given in Table- 2 and 3. It shows that a significant 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) and wet weight of root and shoot (mean value: 0.11 and 0.09 respectively) treated with REEs compared to other trials.

The production stress resistant end product (phenol) is measured using a standard curve obtained from a Folin-Ciocalteau reaction with a phenol solution (C_6H_6O) of 1% and expressed as phenol equivalents in mg/g. The total content of soluble phenols is calculated and given in Tab 4. Seed treated with element alone shows two fold increase in the production of phenol (1000mg/g) compared to others.

Table:3- Measurement of wheat biomass with Rare earth elements along with Pseudomonas sp.

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

The diverse parts of plant samples (root, shoot and leaves) are digested. REE concentrations in the samples are determined by ICP-OES (OPTIMA 5000 DV) analysis Table-5 and Table-6. It reveals that a maximum accumulation of REEs (Cerium and Neodymium) in plant tissues. A higher input of REEs (0.6%) yields high accumulation when compared to lower inputs (0.2% and 0.4%). The seeds treated with culture are shown to have less or no accumulation of REEs by plants.

Table: 4- Assessment of Stress resistant product

S.No	Samples	Optical density (700nm)	Phenols present in plant (mg/g)
1	Seeds without element	0.05	533.33
2	Seeds with element	0.09	1000
3	Seeds without element + culture	0.06	633.33
4	Seeds with element+ culture	0.07	733.33

Table: 5 - ICP-OES Analysis of plant samples with Cerium

S. No.	Sample ID	Sample Composition	REEs accumulation (ppm)	
			X	Y
1	AL	0.2 % element 1+ leaf	0.4	BDL
2	BL	0.4 % element 1+ root	1.1	BDL
3	CL	0.6 % element 1+ leaf	2.7	BDL
4	AR	0.2 % element 1 + root	1.04	BDL
5	BR	0.4 % element 1+ root	5.01	BDL
6	CR	0.6 % element 1+ root	7.08	BDL
7	AS	0.2 % element 1+ shoot	0.91	BDL
8	BS	0.4 % element 1+ shoot	2.1	BDL
9	CS	0.6 % element 1+ shoot	4.9	BDL

A – 0.2%, B – 0.4% and C – 0.6%
L – Leaf, S – Shoot and R – Root
X – Without treatment of bacterial strain
Y – With treatment of bacterial strain
BDL – Below detectable limit (Ce- < 0.0480 mg/L and Nd- < 0.0960 mg/L)

Table: 6 - ICP-OES Analysis of plant samples with Neodymium

S. No.	Sample ID	Sample Composition	REEs accumulation (mg/L)	
			X	Y
1	AL	0.2 % element 2+ leaf	0.04	BDL
2	BL	0.4 % element 2+ root	0.5	BDL
3	CL	0.6 % element 2+ leaf	1.3	BDL
4	AR	0.2 % element 2 + root	0.84	BDL
5	BR	0.4 % element 2+ root	1.87	BDL
6	CR	0.6 % element 2+ root	3.09	BDL
7	AS	0.2 % element 2+shoot	0.92	BDL
8	BS	0.4 % element 2+ shoot	1.36	BDL
9	CS	0.6 % element 2+ shoot	2.04	BDL

A – 0.2%, B – 0.4% and C – 0.6%
L – Leaf, S – Shoot and R – Root
X – Without treatment of bacterial strain
Y – With treatment of bacterial strain
BDL – Below detectable limit (Ce- < 0.0480 mg/L and Nd- < 0.0960 mg/L)

Selective absorption to cell walls may also play a certain role on REE fractionation in the roots, as the REE pattern in the cell walls exhibited slight MREE enrichment. Rare earth elements in plants induce metabolism as a result they are used as fertilizer in agriculture. Metabolism of nutrients in plants increased by rare earth elements by the transfer of N from inorganic to organic form, this is a benefit for protein synthesis and regulation of nutrient balance. Rare earth element in plants enhances the rate of respiration, increases the photosynthesis rate and decreases the loss of water. Spraying rare earth on pepper foliars improved the total chlorophyll content of chl a, chl b (He *et al*., 1998). Rare earth increased the ability of resistance of drought by maize. It also enhance 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.

4. Conclusion

The soil samples were collected from rare earth environments and analyzed by ICP-MS to know constituents of the rare earth elements. Cerium and Neodymium are abundant in the analyzed samples. The physical parameters of the wheat biomass are evaluated (root and shoot length, wet and dry weight of root and shoot) with and without treatment of REEs and also treated with REEs and a bacterial strain (*Pseudomonas sp.,*). From the results it is evident that a significant increase in root and shoot length, dry weight and wet weight of plants treated with REEs. The production stress resistant end product (phenol) is measured and the total content of soluble phenols is calculated. Seeds treated with element alone show two fold increases in the production of phenol. The diverse parts of plant samples (root, shoot and leaves) are digested. REE concentrations in the samples are determined by ICP-OES analysis. It reveals that a maximum accumulation of REEs (Cerium and Neodymium) in plant tissues and higher input of REEs yields high accumulation and lower inputs yield low accumulation. The seeds treated with culture are shown drastic change in the accumulation of REEs by wheat (*Triticum aesativum*).

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