DEPENDANCE OF IRON UTILIZATION BY RICE ON NITROGEN SOURCE IN THE GROWTH MEDIUM

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ABSTRACT

When rice plants were grown in sand culture with ammonium or ammonium nitrate as source of nitrogen, they could utilise all sources of iron almost equally well, but when the source of nitrogen supply was nitrate or urea they showed differential effect of the form of source of iron supply. As judged by visual effects, depression in growth and chlorophyll and in the activities of catalase and peroxidase, plants receiving nitrate or urea could utilise ferric—EDTA but could not utilise ferric chloride, ferric citrate and ferric-EDDHA and could very poorly utilise ferrous sulphate and ferric tartrate. The growth with ferric-EDTA as source of iron and nitrate and urea as sources of nitrogen was still depressed as compared to plants grown with ammonium or ammonium nitrate.

INTRODUCTION

During the course of studies undertaken to resolve some nutritional disorders in rice plants under field conditions, it was observed that when grown with standard culture solution (HEWITT, 1952), where nitrogen is provided as nitrate and iron as ferric citrate, rice plants showed iron chlorosis and depression in growth. This was contrary to the results obtained by GERICKE (1930) who found that with nitrate nitrogen, ferric citrate and ferric tartrate were good sources of iron but inorganic forms of iron like ferric chloride, ferric phosphate, and ferrous sulphate were not suitable iron sources; the inorganic forms of iron were found to be suitable only when the source of nitrogen was ammonium. SIRKAR AND DUTTA (1957) could grow rice with ferric chloride as source of iron when the source of nitrogen was nitrate. In view of the conflicting results obtained by different workers it was considered advisable to examine which form of iron-ferric chloride, ferrous sulphate, ferric citrate, ferric tartrate, ferric-EDTA and ferric EDDHA—would be suitable with the different common sources of nitrogen—nitrate, ammonium sulphate, ammonium nitrate and urea.

MATERIAL AND METHODS

Rice (*Oryza sativa* L. var. IR. 8) plants were grown in silica sand purified by the technique of HEWITT (1952) as adapted for Indian conditions (AGARWALA & SHARMA, 1961) in 9" clay pots painted thrice with bitumen. The pots contained a central drainage hole which was covered with a watch glass and glass wool such that water could flow freely through it. The composition of the nutrient solution used for growing plants is described in Table 1.

Nitrogen was supplied at 15 m.eq./L in the form of sodium nitrate, ammonium sulphate, ammonium nitrate, or urea. For each form of nitrogen supply iron was supplied at 11.2 ppm as ferric citrate, ferrous sulphate, ferric tartrate, ferric chloride, ferric-EDDHA (ferric ethylenediamine (di) o-hydroxyphenyl acetic acid) or ferric-EDTA (ferric ethylenediamine tetraacetic acid). In addition, there was a treatment in which iron supply was

Table 1—Composition of nutrient solution used for the culture of rice.

Nutrient element	*Level of supply*	*form in which supplied*
	m.eq./L	
Potassium	4	Sulphate
Calcium	8	Sulphate
Phosphorus	4	Sodium salt (NaH_2PO_4)
Sulphur	15	Sulphate of K, Ca and Mg
Sodium	1.33	Sodium salt (NaH_2PO_4)
Magnesium	4	
	ppm	Sulphate
Manganese	0.55	Sulphate
Copper	0.065	,,
Zinc	0.065	,,
Molybdenum	0.05	Sodium salt
Boron	0.37	Boric acid
Cobalt	0.006	Sulphate
Nickel	0.006	,,
Nitrogen and iron	As indicated in text	

withheld (—Fe). Ferric-EDTA was prepared by the method of JACOBSON (1951) and ferric citrate as described by HEWITT (1952). Fe-EDDHA used in the studies was obtained from Ms J. R. Giegy, A. G., Basel, Switzerland. Other forms of iron were supplied as A. R. grade salts commercially available. The macro-nutrient solutions were purified against iron by phosphate adsorption technique (HEWITT, 1952). A. R. grade salts were used for supplying micronutrients.

Plants grown at differential nitrogen-iron treatments were assayed for dry matter yield, chlorophyll, tissue iron, nitrate and protein nitrogen and for the activity of catalase and peroxidase. Dry matter yield and chlorophyll were determined at two stages—21 and 32 days growth. The other determinations were made at one stage—21 days growth. Dry matter was determined by drying the plant material, which had been rinsed with dilute hydrochloric acid and then with deionised water, in a forced draught oven at 70°C for 24 hours. Chlorophyll was determined by the method of PETERING, WOLMAN AND HIBBARD (1940). The calibration curve for chlorophyll was prepared by the method of COMAR AND ZECHEILE (1942). Iron was determined in the oven-dried plant material as ferrous—O phenanthroline complex (HUMPHRIES, 1956) after wet digestion with nitric and perchloric acids (PIPER, 1942).

Catalase and peroxidase were assayed in crude tissue extracts. The fresh material for the estimation of the two enzymes was finely chopped, chilled and ground with acid washed sand in a chilled pestle and mortar in 0.005 M phosphate buffer pH 7 in the proportion of 1 g leaf material to 10 ml of the buffer. Grinding was carried out in an ice bath. The crude extract was strained through two fold muslin, and catalase and peroxidase were assayed within 3 hours of the preparation of the extract. Catalase was assayed by an adaptation of the method of EULER AND JOSEPHSON (1927) and peroxidase by the method of WILLSTATTER AND STOLL as modified by SUMNER AND GJESSING (1943). The amount of the enzymes for the assay was taken in the range in which their activity was proportional to their concentration.

Nitrate nitrogen was estimated by the method suggested by RICHARD AND TEMPLEMAN (1936). Protein nitrogen was determined in the alcohol insoluble material digested according to the procedure of CHIBNALL, REES AND WILLIAMS (1943). The ammonia produced

was distilled in a Markham distillation apparatus into boric acid buffer and estimated by titration with N/140 sulphuric acid containing Conway O'Malley indicator (Conway & O'Malley, 1942).

Observations

Visual Effects

Within fifteen days of the sowing of seeds, chlorosis developed in foliage of rice plants raised with nitrate or urea where the form of iron supply was ferric citrate, ferric-EDDHA or ferric chloride. Within next five days chlorosis became marked in these treatments and mild chlorosis also developed in plants receiving nitrate or urea and iron in the form of ferrous sulphate, ferric tartrate and Fe-EDTA. Later on, at about 28 days growth, chlorosis became marked in all plants receiving nitrate or urea and iron in any form except Fe-EDTA. In addition, plants receiving these treatments developed necrosis and collapsed. Plants grown with ammonium sulphate or ammonium nitrate did not show chlorosis except in the begining and this soon disappeared resulting in normal healthy plants.

Dry Weight Yield

As compared to plants receiving ammonium sulphate or ammonium nitrate, plants receiving nitrate or urea showed a marked reduction in dry weight yield. The source of iron supply did not very much affect the yield of plants when the source of nitrogen supply was ammonium sulphate or ammonium nitrate except that irrespective of the source of iron supply the yield was higher with ammonium nitrate than with ammonium sulphate (Table 2). But the source of iron supply had a marked effect on the yield of plants when

Table 2—Effect of nitrogen and iron sources on the dry weight yield of rice var. IR8 plants grown in sand culture

Nitrogen source	Iron source							
	—Fe	Ferrous Sulphate	Ferric citrate	Ferric tartrate	Ferric EDTA	Ferric EDDHA	Ferric chloride	L.S.D. (P= 0.05)
	mg dry weight per plant : 21 days growth							
Nitrate	7	31	21	42	59	19	17	
Ammonium nitrate	9	125	121	128	125	122	118	7
Ammonium sulphate ..	9	125	119	120	126	122	122	
Urea	7	35	27	31	73	19	20	
	mg dry weight per plant : 32 days growth							
Nitrate	Plants dead	34	47	48	174	39	29	
Ammonium nitrate	540	570	563	566	580	555		47
Ammonium sulphate	405	421	390	400	383	376		
Urea	30	31	40	233	50	21		

the source of nitrogen was nitrate or urea. Both at 21 days and 32 days growth, yield was highest in the nitrate and urea treatments when the source of iron supply was Fe-EDTA (Table 2). At 21 days growth, the yield in the other iron treatments was in the decreasing order ferric tartrate, ferrous sulphate, ferric citrate, ferric-EDDHA and ferric chloride. These differences, however, narrowed down later and disappeared at 32 days growth.

Table 3—Effect of nitrogen and iron sources on the chlorophyll content of rice var. IR. 8 plants grown in sand culture

Nitrogen Source	Iron Source							
	—Fe	Ferrous sulphate	Ferric citrate	Ferric tartarate	Ferric EDTA	Ferric EDDHA	Ferric chloride	P.D.
	mg. chlorophyll per 100 g. fresh weight : 21 days growth							
Nitrate	13	63	33	58	83	31	37	
Ammonium nitrate	15	123	118	116	120	109	112	18
Ammonium sulphate ..	18	115	119	113	118	121	115	
Urea	11	65	40	54	80	38	37	
	mg. chlorophyll per 100 g. fresh weight : 32 days growth							
Nitrate	21	18	20	79	17	13		
Ammonium nitrate	119	125	119	121	124	117		28
Ammonium sulphate	115	118	123	109	114	115		
Urea	23	23	17	76	15	9		

Chlorophyll

Irrespective of the source of nitrogen supply chlorophyll was greatly depressed in plants grown without iron (Table 3). When the form of nitrogen supply was ammonium sulphate or ammonium nitrate, the form of iron supply did not make any difference in the chlorophyll content of rice plants. But when the form of nitrogen supply was nitrate or urea, the form of iron supply made a good deal of difference in the chlorophyll content of rice plants, the effects being more at 21 days than at 32 days growth. With nitrate or urea as compared to the other iron treatments, chlorophyll content was higher in plants supplied iron as ferric-EDTA. These values of chlorophyll were, however, appreciably lower than in corresponding plants receiving nitrogen as ammonium sulphate or ammonium nitrate (Table 3). At 21 days growth, the decrease in the chlorophyll content of plants receiving nitrate or urea was less marked if plants received iron as ferrous sulphate and ferric tartrate than when iron was supplied as ferric citrate, ferric chloride and ferric-EDDHA.

Tissue iron

Neither did iron deficiency depress the iron concentration of rice plants, nor was tissue iron consistently related to the source of iron or nitrogen supply (Table 4).

Catalase

In plants grown without iron, catalase was very much depressed irrespective of the form of nitrogen supply. With ammonium sulphate and ammonium nitrate the source

Table 4—Effect of nitrogen and iron sources on the tissue concentration of iron in rice var. IR 8 plants grown in sand culture : 21 days growth

Nitrogen Source	Iron source							
	—Fe	Ferrous sulphate	Ferric citrate	Ferric tartarate	Ferric EDTA	Ferric EDDHA	Ferric chloride	L.S.D. (0.05)
					μg iron per g dry weight in top parts			
Nitrate	115	109	79	87	78	89	103	
Ammonium nitrate	107	93	119	94	97	115	101	33
Ammonium sulphate ..	121	87	79	112	102	96	113	
Urea	115	115	119	94	130	245	104	
					μg iron per g weight in roots			
Nitrate	311	278	278	385	206	330	361	
Ammonium nitrate	370	283	450	391	293	600	375	73
Ammonium sulphate ..	350	304	402	370	300	350	344	
Urea	390	385	466	366	457	663	375	

of iron supply did not make any marked difference in the catalase activity but when the source of nitrogen supply was nitrate or urea the source of iron supply had a differential effect, the catalase activity being the highest with Fe-EDTA as compared to the other iron treatments (Table 5). The other iron treatments, when the source of nitrogen was nitrate

Table 5—Effect of nitrogen and iron sources on the activity of catalase and peroxidase in crude tissue extracts of rice var. IR 8 plants grown in sand culture

Nitrogen Source	Iron source							
	—Fe	Ferrous sulphate	Ferric citrate	Ferric tartrate	Ferric-EDTA	Ferric-EDDHA	Ferric chloride	L.S.D. (0.05)
					Catalase : μ moles H_2O_2 split per mg protein nitrogen			
Nitrate	17.85	34.48	22.79	31.79	58.98	40.34	19.0	
Ammonium nitrate	19.33	81.59	82.07	86.63	89.02	107.61	87.28	10.3
Ammonium sulphate ..	17.74	80.40	84.78	91.91	83.33	95.95	98.98	
Urea	13.15	31.79	20.64	33.70	67.64	23.64	23.39	
					Peroxidase : mg. purpurogallin formed per mg protein nitrogen			
Nitrate	4.75	4.48	3.98	4.42	5.28	3.45	4.67	
Ammonium nitrate	4.66	5.08	5.47	5.80	5.19	6.07	5.03	1.0
Ammonium sulphate ..	4.77	4.89	5.48	5.26	5.47	5.55	4.27	
Urea	4.44	4.97	4.86	4.95	5.64	3.83	4.23	

or urea, could be arranged in two groups, ferric chloride, ferric citrate and ferric-EDDHA having the most marked depression and ferrous sulphate and ferric tartrate having less marked depression.

Peroxidase

In plants grown without iron, peroxidase was depressed irrespective of the source of nitrogen supply (Table 5). In plants grown with ammonium sulphate and ammonium nitrate the iron source did not affect the peroxidase activity but in plants given nitrate or urea the peroxidase activity was lower than in plants given ammonium sulphate or ammonium nitrate. In the nitrate or urea treatments iron supply also made a difference in the peroxidase activity of the rice plants, the activity of the enzyme being higher in plants supplied iron as Fe-EDTA than in plants supplied iron in the other forms (Table 5).

Nitrate and Protein nitrogen

There was greater accumulation of nitrate in plants supplied nitrogen as nitrate than in plants supplied nitrogen in the form of ammonia, ammonium nitrate or urea (Table 5).

Table 6—Effect of nitrogen and iron sources on the nitrate and protein nitrogen content of rice var. IR 8 plants grown in sand culture

Nitrogen source	Iron source							
	—Fe	Ferrous sulphate	Ferric citrate	Ferric tartarate	Ferric EDTA	Ferric EDDHA	Ferric chloride	S.D. 0.05
	percent nitrate in dry matter : 21 days growth							
Nitrate	0.328	0.230	0.208	0.120	0.133	0.230	0.270	..
Ammonium nitrate	0.092	0.045	0.098	0.095	0.090	0.092	0.096	..
Ammonium sulphate	0.070	0.040	0.025	0.095	0.045	0.030	0.120	..
Urea	0.065	0.040	0.057	0.060	0.035	0.060	0.056	..
	mg protein nitrogen per 100 mg fresh weight							
Nitrate	2.8	3.48	3.51	3.46	3.56	3.47	3.42	.048
Ammonium nitrate	3.00	4.02	3.96	4.04	4.10	4.20	4.01	..
Ammonium sulphate ..	3.10	3.98	4.01	4.08	4.20	3.96	3.94	..
Urea	3.04	3.46	3.39	3.56	3.40	3.40	3.42	..

With nitrate as the source of nitrogen, nitrate accumulation was most marked in plants grown without iron, less so with ferrous sulphate, ferric citrate and Fe-EDDHA and the least with ferric tartrate. With other sources of nitrogen the effect of iron source was not so marked. Perusal of nitrate content of plants grown with different forms of nitrogen (Table 6) suggested that a part of the ammonia and urea was also converted to nitrate. Protein nitrogen was depressed in plants grown without iron irrespective of the nitrogen source. It was also depressed in plants grown with nitrate or urea compared to plants grown with ammonium sulphate or ammonium nitrate.

DISCUSSION

As has been reported by GERICKE (1930) and ASANA (1945) we found that almost all iron sources were suitable for the growth of rice when the source of nitrogen was ammonium; ferric chloride being a little less suitable than the other forms of iron. With nitrate nitrogen the results obtained here are not in accord with those of SIRKAR AND DATTA (1957) regarding the suitability of ferric chloride, with those of GERICKE (1930) regarding the suitability of ferric citrate and with those of ASANA (1945) regarding the suitability of ferric tartrate and ferrous sulphate as iron source for the growth of rice, Surprisingly, Fe-EDDHA, which according to WALLACE (1963) appeared to be the best iron source in nutrient solutions (hydroponics), was found to be a very poor iron source when the nitrogen source was nitrate or urea. Amongst the sources tried Fe-EDTA proved to be the best iron source with nitrate nitrogen. Urea also behaved like nitrate.

As has been reported by other workers (EPSINO & ESTIOKE, 1931; THELIN & BEAUMONT, 1934; DASTUR & MALKANI, 1933; DASTUR & KALYANI, 1934; ASANA, 1945) a combination of ammonium and nitrate nitrogen was found to be a better source of nitrogen than ammonium or nitrate nitrogen alone.

The relation of the form of iron supply to that of the form of nitrogen supply can neither be attributed to the differential uptake of nitrogen nor to that of iron. The iron content in the treatments in which of plant growth was depressed was in many cases higher than in those in which growth was normal. Irrespective of the nitrogen source, plants from which iron supply was withheld contained a higher concentration of iron than in plants which had been supplied iron. Earlier, AGARWALA AND SHARMA (1961a) have drawn attention to a large number of cases wherein tissue concentration of iron was higher in plants showing severe iron deficiency effects than in the plants showing normal healthy growth. Similar observations have also been made by AGARWALA, SHARMA AND KUMAR (1964), AGARWALA, SHARMA AND FAROOQ (1965), BRANTON AND JACOBSON, (1962), and MARSH, EVANS AND MATRONE (1963).

It was observed that not only plants supplied nitrogen as nitrate accumulated nitrate but plants supplied nitrogen as ammonium or urea also accumulated it. This suggested that under the culture conditions urea and ammonium nitrogen were being partially converted into nitrate. Somewhat greater accumulation of nitrate in iron deficient plants than in those to which iron was supplied as ferric citrate or ferric chloride would, at first sight, suggest that iron was directly involved in the utilization of nitrate. But as the magnitude of accumulation of nitrate was nothing like that encountered in molybdenum deficiency (AGARWABA, 1952; AGARWALA & HEWITT, 1955), where molybdenum is the prosthetic group of nitrate reductase, it appears unlikely that iron may be a co-factor in the reduction of nitrate. But this possibility can not be totally discounted and needs further examination, particularly in view of the fact that iron in the form of cytochrome has been shown to be involved in the activity of nitrate reductase of some bacteria (SATO & NIWA, 1952; BAALSRUD & BAALSRUD, 1954; KAMEN & VERNON, 1955; LENHOF, NICHOLAS & KAPLAS, 1956; KINSKY & MCELROY, 1958; FEWSON & NICHOLAS, 1960; WALKER & NICHOLAS, 1961) and in bean seedlings (EGAMI, CHAMACHI, LIDA & TANAGUCHI, 1957). VAIDYANATHAN AND STREET (1959) reported a nitrate reductase from tomato roots requiring ferrous iron and ascorbic acid as essential co-factors.

The protein nitrogen was found to be depressed in plants from which iron supply was withheld and in those iron treatments in which with nitrate or urea nitrogen growth was depressed. Several other workers have also reported a decrease in the protein content of iron deficient plants (WEINSTEIN & ROBBINS, 1955; PERUR, SMITH & WIEBE, 1961;

Welkie & Miller, 1960; Shetty & Miller, 1966). It could well be that, as in maize and radish (Agarwala, Sharma & Farooq, 1965), iron is involved in protein synthesis in rice also.

The results presented here unequivocally show that utilization of iron in rice is dependent on the form in which nitrogen was supplied. Thus chlorophyll and catalase were depressed in rice plants to which nitrogen was supplied either as nitrate or urea and the source of iron was ferric chloride, ferric citrate or ferrous sulphate—treatments in which growth was depressed. Further studies are in progress to ascertain whether the results reported here are an outcome of an interaction of iron and nitrogen in the rooting medium or on the root surface, or due to impeded uptake of iron. These studies using radioactive iron will be reported elsewhere.

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