热带亚热带植物学报　1995, 3(4):49－55
Journal of Tropical and Subtropical Botany

亚铁离子对水稻萌发后幼苗生长的促进作用

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摘要　本文最新报道亚铁离子在远高于营养浓度的情况下(0.44－7.16mmol/L)能显著促进水稻萌发后的幼苗生长，其效果与已报道的促进因子C_2H_4相当．在试验的19种不同化合物中，发现只有$FeSO_4$对萌发后水稻幼苗生长有显著的促进作用，并证明其有效成分为Fe^{2+}．这种促进作用在淹水的厌氧条件下和通气条件均能发生，但通气条件下促进程度稍低．水稻不同基因型对Fe^{2+}的反应无明显差异，可能这种促进作用是水稻适应水田厌氧环境的方式之一．高浓度乙烯(50－100ppm)的促进作用与亚铁离子的效果相近．这一发现有助于提高水稻在淹水厌氧直播时的出苗率．

关键词　亚铁离子；水稻幼苗；淹水；乙烯

STIMULATION OF THE SEEDLING GROWTH OF RICE BY FERROUS ION

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Abstract The paper reports a new finding that Fe^{++} with high concentrations (0.44－7.16 mmol/L) is another effective stimulating factor other than the reported ones like CO_2, ethylene, etc. Under submergence, 4 out of 19 tested chemicals, at the same concentration of 1.79mmol/L, inhibited the seedling growth of rice, only $FeSO_4$ significantly stimulated it, and the other ones had no effect. In aerobic condition, this stimulation still occur, but with a smaller extent. No obvious genotype difference was observed in the response to ferrous ion, which might imply that this stimulation behavior is one of the ways for rice to adapt flooded condition. High concentration of ethylene had a similar stimulative effect like Fe^{++} on the seedling growth of rice. This finding may probably help to improve the seedling establishment in the direct seeding, in which germinated seeds are sowed under flooded soil surface.

Key words Ferrous ion; Rice seedling; Submergence; Ethylene

1995－02－28 收稿；1995－04－24 修回

In tropical rice production, direct seeding is becoming more and more popular than the traditional method of transplanting because it uses less labor and entails low cost. A new method of direct seeding is recently being developed, in which germinated seeds are sowed under flooded soil surface so as to avoid some constraints usually encountered in the old method of direct seeding, in which germinated seeds are broadcast onto the soil surface after drainage[1]. When germinated seeds are sowed under flooded soil surface, the seedling establishment is relatively poor mainly because the growth in this case is restricted to coleoptile and mesocotyl elongation while leaf and root growth is severely inhibited due to lack of oxygen[2,3], which is also the main problem for this new method. But as an adaptive behavior, rice coleoptile has the potential capacity to elongate until it reaches an aerobic layer from which oxygen can be transferred to the seeds, with subsequent normal growth of radicle and leaf[4,5]. Thus, a fast and vigorous coleoptile and mesocotyl elongation under flooded soil surface is of special importance for the seedling establishment in this new technology of direct seeding.

The coleoptile elongation is stimulated by low O_2 and high CO_2 concentration or by ethylene[5-9]. Those factors appear to contribute equally to the stimulation of the coleoptile growth, and each gas can promote growth independently of the others[8]. Recently, the role of polyamines in the anaerobic elongation of rice coleoptile was determined, showing that the coleoptile elongation was massively stimulated by the polyamines under anaerobic conditions[10]. In addition, unknown factor(s) is thought to be involved in the growth of coleoptile under water[11].

This paper reports our finding that ferrous ion with high concentrations (0.44-7.16 mmol/L) is an effective stimulating factor to the seedling growth of rice under both aerobic and anaerobic conditions.

1 Materials and methods

1.1 Seeds

Seeds, supplied by the International Rice Germplasm Center, the International Rice Research Institute, were multiplied in 1992 and 1993. Dormancy was broken by keeping the seeds at 50 ℃ for 5 days. Seeds were then stored at below 5 ℃ until use.

1.2 Incubation in submerged system

Seeds were sterilized by immersion in 1% hypochlorite solution for 15 min, and then washed thoroughly and soaked in distilled water for 2h. Six hundred of seeds were placed in a petri dish (diameter 14cm) lined with 3 layers of filter paper holding 15ml distilled water, then germinated in the dark at 30 ℃ for 2 days.

The germinated seeds were transferred to Pyrex Erlenmeyer flasks (inner volume 33 ± 0.3 ml) with 30 seeds per flask. Certain amount of distilled water or test solution was added to the flask up to 4.5cm of depth for each solution. The flask was then wrapped and covered loosely by aluminum foil to maintain darkness thereafter. The flasks were incubated for 3 days at 30 ℃ in a water bath.

1.3 Incubation in aerated system

Thirty 2-day pregerminated seeds (as above described) were transferred to a flask containing 3ml of distilled water or test solution. The flask was then plugged with serum bottle stopper (sleeve type), and wrapped in aluminum foil. The humidified air was introduced at a rate of 0.5L min^{-1} to the flask for 1 min each day.

1.4 Growth measurement

The lengths of the coleoptile, mesocotyl, shoot and root were measured with a ruler after incubation for different days. At the start of incubation, initial coleoptile and root lengths were also measured.

1.5 Statistical analysis

The flasks for incubation were laid out in a completely randomized design with three replications. The microcomputer program IRRISTAT[12] was used for the mean comparison.

2 Results

2.1 Effect of some chemicals on the seedling growth of rice

Nineteen kinds of chemicals, which cover nutrient elements and the chemicals usually existing in flooded problem soil, were tested about their effects on the seedling growth of rice. Under submergence and at 1.79mmol/L of concentration, $CuSO_4$, NH_4NO_3, KNO_3 and NH_4HCO_3 inhibited the growth of coleoptile and mesocotyl, while $FeSO_4$ significantly stimulated it. The other chemicals had no effect (Table 1). At as much as the concentration in normal nutrient solution[13], all chemicals had no any effect on the growth under submergence (data not shown).

2.2 Growth response of some genotypes to ferrous ion under submergence

The growth response of 9 genotypes to ferrous ion was tested under submergence. The coleoptile elongation of all tested genotypes were stimulated by Fe^{++}, no regular difference could be observed among the tested genotypes. The root growth was not influenced by Fe^{++} under submergence (Table 2).

Table 1 Effect of some chemicals on the seedling growth of rice under submergence

Chemical	Length of treatment / Length of control			Chemical	Length of treatment / Length of control		
	Coleoptile	Mesocotyl	Root		Coleoptile	Mesocotyl	Root
NH_4NO_3	0.49**	0.27**	1.17	Fe (III) EDTA	1.02	1.12	0.98
NaH_2PO_4	1.01	0.90	0.93	KNO_3	0.68*	0.70*	1.15
K_2SO_4	1.09	1.15	0.93	NH_4HCO_3	0.84*	0.83	1.18
$CaCl_2$	0.96	0.93	1.06	KH_2PO_4	1.07	0.84	1.02
$MgSO_4$	0.85	0.82*	1.01	NaCl	0.90	0.83	1.15
$MnCl_2$	0.90	0.78*	0.96	KCl	0.94	0.99	1.12
Na_2MO_4	1.02	1.11	1.07	$(NH_4)_2SO_4$	1.05	1.27	0.85
H_3BO_3	0.95	0.96	1.16	$MgCl_2$	0.90	0.78	0.96
$ZnSO_4$	1.08	1.17	0.99	$FeSO_4$	1.63**	1.68**	1.00
$CuSO_4$	0.45**	0.79**	0.98				

The concentration of the chemicals was 1.79mmol/L. pH of solutions was adjusted to 5.5.
*— significant at 5% level; **— significant at 1% level

Table 2 Growth response of some genotypes to Fe^{++} under submergence

Genotype	Length in Fe^{++}/Length in H_2O			Genotype	Length in Fe^{++}/Length in H_2O		
	Coleoptile	Mesocotyl	Root		Coleoptile	Mesocotyl	Root
IR50	1.70**	1.64*	0.99ns	Taothabi	1.16**	1.41**	0.99ns
ASD1	1.28**	2.33**	0.96ns	JC178	1.48**	1.42**	0.96ns
IGR183	1.17*	1.21ns	0.98ns	PSBRc4	1.90**	1.51ns	0.99ns
IR72	1.50**	1.69*	0.88ns	Kuhei	1.28**	1.31ns	1.28ns
IR36	1.29**	1.22ns	0.97ns				

Fe^{++} concentration was 1.79mmol/L (pH 5.5). **— significant at 1% level;
*— significant at 5% level; ns — not significant.

2.3 Effect of various Fe^{++} concentrations on the seedling growth of rice

Two genotypes, modern IR50 and traditional ASD1, were used for further study. Fe^{++} with concentrations from 0.44 to 7.16mmol/L was able to stimulate the coleoptile and mesocotyl elongation of both genotypes under submerged or aerated condition (Fig. 1 and Fig. 2). Under submergence no leaf was found and the root was also unable to grow any more (Fig. 1C). Although the roots in aerated condition can keep growing, but they were not stimulated by Fe^{++}, and even inhibited when the concentration was over 1.79mmol/L (Fig. 2C).

2.4 Time course of the stimulation by Fe^{++}

In submerged condition, the coleoptile growth of IR50 and ASD1 were almost linearly increased within 3-day incubation for both treated and untreated seedlings. The coleoptile elongation rates of control and treated IR50 seedlings were 5.3mm per day and 8.3mm per day, respectively. The rate of treated ASD1 was 10.0mm per day in contrast to

6.9mm per day of untreated one (Fig. 3A). In aerated condition, the stimulation extent by Fe^{++} was more than 20% smaller than that in submerged condition (Fig. 3B).

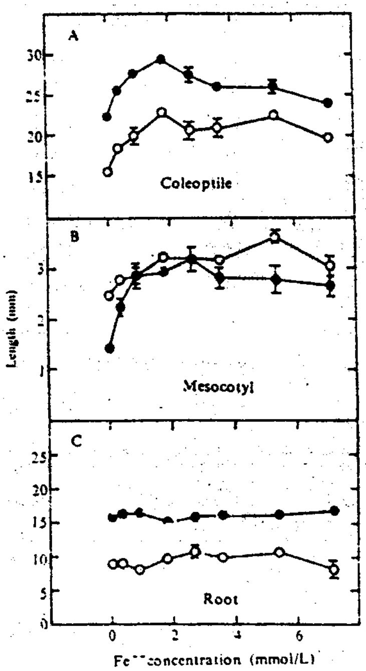

Fig. 1　Effects of various concentrations of Fe^{++} on the seedling growth of rice under submergence

○—○ IR50;　●—● ASD1

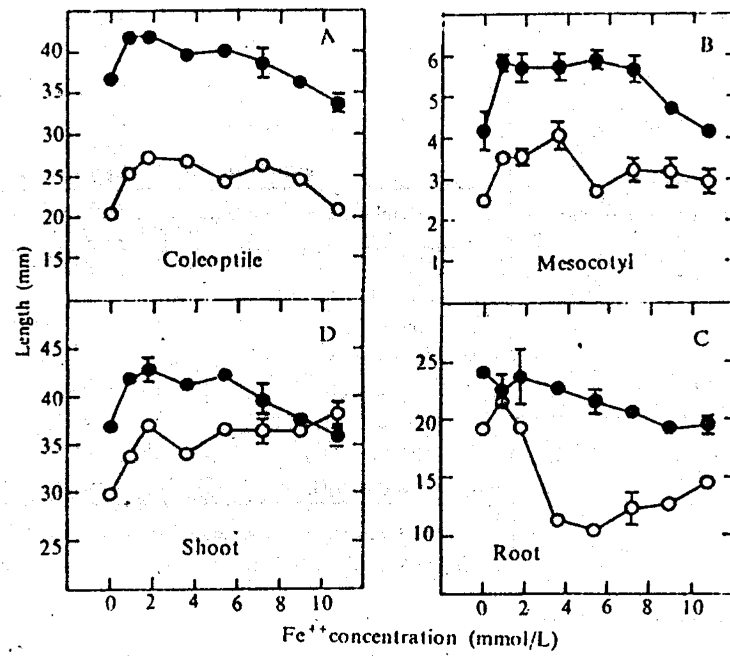

Fig. 2　Effects of various concentrations of Fe^{++} on the seedling growth of rice in aerated condition

○—○ IR50;　●—● ASD1

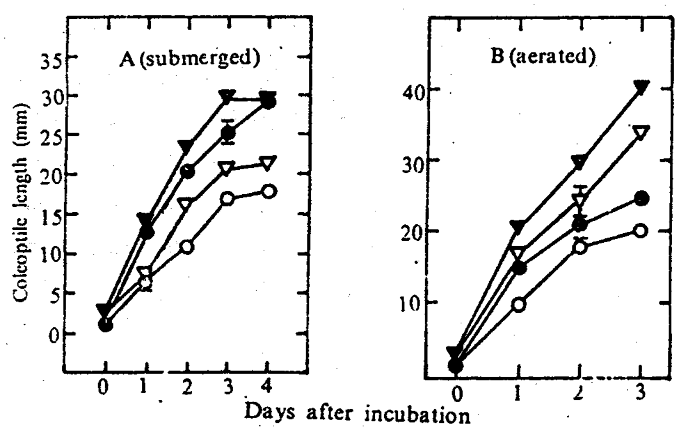

Fig. 3　Time course of the stimulation of rice coleoptile growth by Fe^{++} under submerged and aerated conditions

○—○ IR50 in water;　●—● IR50 in 1.79mmol/L Fe^{++}

△—△ ASD1 in water;　▲—▲ ASD1 in 1.79mmol/L Fe^{++}

2.5 Interaction between Fe^{++} and ethylene in stimulating the seedling growth

Fe^{++} and exogenous ethylene singly stimulated the growth of coleoptile, shoot and mesocotyl. If low concentration of ethylene (10ppm) was applied together with Fe^{++}, they stimulated the seedling growth additively. When ethylene concentration was increased over 50ppm, its effectiveness is similar to that of Fe^{++}. (Table 3).

Table 3 Interaction between Fe^{++} and ethylene in regulating the seedling growth of rice in aerated condition

Combination	Length (mm)			
	Coleoptile	Shoot	Mesocotyl	Root
H_2O	20.12c	31.67b	1.46c	23.14ab
Fe^{++}(1.79mmol/L)	27.94ab	39.79a	2.58b	23.75a
10ppm C_2H_4	25.56b	31.69b	2.57b	23.49ab
10ppm C_2H_4 + Fe^{++}	30.63a	44.66a	3.33a	20.61ab
50ppm C_2H_4	27.94ab	39.55a	2.33b	22.27ab
50ppm C_2H_4+Fe^{++}	31.10a	42.55a	3.52a	21.23ab
100ppm C_2H_4	29.11ab	38.59a	2.64b	21.50ab
100ppm C_2H_4+Fe^{++}	30.72a	43.37a	3.78a	19.12b

Means followed by a common letter in each column are not significantly different at the 5% level by DMRT

3 Discussion

In our screening experiment, the genotypes screened from anaerobic soil condition were inconsistent with those screened in N_2 flushed water condition (data not shown), we speculated that some other factor(s), except those mentioned previously[8], may control the seedling growth of rice in soil anaerobic condition. Ishizawa and Esashi[11] also pointed out that unknown factor(s) other than O_2, CO_2, C_2H_4 were involved in the enhanced elongation of rice coleoptile under water. We tested many kinds of chemical elements usually existing in flooded soil. The result showed that only high concentration of $FeSO_4$ may significantly stimulate coleoptile and mesocotyl elongation (Table 1 and Fig. 1), no stimulative effect was observed when those elements were at a nutrient concentration (data not shown). Meanwhile as judged from Table 1, the effective element in $FeSO_4$ is ferrous ion. High concentration of ferrous ion is very toxic to plants even though it is an essential element for plant growth. Iron toxicity usually occurs in flooded problem soils[14]. The present result is unexpected but very interesting and meaningful. Practically, in the new method of direct seeding we might be able to improve the seedling growth by choosing the right sowing time after flooding the soil because a certain profile of Fe^{++} concentration may be determined experimentally, depending on the different types of soil[14]. On the other hand, this stimulation of seedling growth of rice by Fe^{++} may be one of the ways for rice to adapt flooded condition since there was no obvious genotype difference in response to ferrous ion (Table 2). How high concentration of Fe^{++} can stimulate the seedling growth of rice is still unclear. As seen from Table 3, high concentration of exogenous ethylene showed the similar effect like Fe^{++} on the seedling growth. Whether or not the stimulation by Fe^{++} is mediated by ethylene is being investigated. If it is true, why the stimulation extent by Fe^{++} under submerged condition was greater than that in aerated condition can be explained as follows: under submergence, endogenous ethylene diffuses out to the ambient environment more slowly than it does in aerated condition, and then it may accumulate locally inside the tissue to cause a more effective stimulation.

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