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水稻铁害和应激乙烯释放的关系

彭新湘 Minoru Yamauchi
（华南农业大学生物系，广州 510642） （菲律宾国际水稻研究所）

摘要 在热带地区的水稻栽培中，常遇到水稻青铜病(bronzing)的危害．已知它是由水田中高浓度的亚铁离子所引起，故又叫铁害．但至今没有可靠的生理诊断指标用于抗性品种的筛选．本文研究了铁害与应激乙烯释放的关系，试图以应激乙烯的释放作为铁害的生理诊断指标．试验用两种方法模拟水稻致病．第一种方法是将水稻离体叶片的剪口端浸入$FeSO_4$溶液中，靠叶片蒸腾作用吸收Fe^{++}而致病．另一种方法是在水培培养液中加入$FeSO_4$，通过水稻根系吸收Fe^{++}而致病．研究结果表明，当处理离体叶片时，发病强度和应激乙烯释放量呈显著相关，但叶片内铁含量的增加与发病强度和应激乙烯释放都没有相关性．而处理完全植株时，叶片中乙烯释放几乎不受影响．当部分或全部切除根时，叶片中乙烯释放则可被亚铁离子激发．表明水稻根系限制了Fe^{++}的吸收速率，而Fe^{++}进入叶组织的速率又决定应激乙烯的释放和组织的伤害程度．因此，叶片应激乙烯的释放作为铁害的生理诊断指标只有在当根系受到某种伤害时才可能适用，譬如移栽和毒性土壤等因素造成的根系的伤害．

关键词 应激乙烯；铁害；水稻

IRON TOXICITY AND STRESS ETHYLENE PRODUCTION IN RICE

Peng Xinxiang
(South China Agricultural University, Guangzhou 510642)
Minoru Yamauchi
(International Rice Research Institute, P. O. Box 933, 1099 Manila, Philippines)

Abstract The relationship between Fe^{++}-induced bronzing and stress ethylene production (SEP) was investigated both in detached rice (*Oryza sativa* L.) leaves and in whole plants. Fe^{++} was applied to the detached leaf through a transpiration stream and to whole plants through roots in culture solution. The SEP from detached leaves differed with leaf position, growth stage, and genotype. Correlation between SEP and bronzing was significant for the leaves of 16 tested genotypes ($r=0.659^{**}$), but the iron concentration increment (ICI) of the detached leaf correlated neither to the bronzing nor to the SEP, suggesting that leaf tissue tolerance, not ICI, controls the bronzing development and the SEP. When the whole plant was treated by increasing Fe^{++} concentration in culture solution, the SEP was negligibly small. By partially or entirely de-rooting the plant, however, stress ethylene was evoked by

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the Fe^{++} treatment, indicating that roots limited the Fe^{++}-induced SEP.

Key words Stress ethylene; Iron toxicity; Rice

Iron toxicity is a nutritional disorder of rice plants associated with high Fe^{++} level in flooded soil, which is widely distributed in tropical lowlands. The leaf symptom characterized by bronzing and yellowing is usually used to indicate the toxic intensity.

Genotype difference in tolerance for iron toxicity is thought to be caused by the difference in Fe^{++} oxidation in rhizosphere[1-3]. However, Jayawardena *et al.*[4] failed to find any correlation between plant tolerance, leaf iron concentration, and root α-naphthylamine oxidizing power, and suggested that genotype difference in the tolerance was attributed to the leaf tissue tolerance rather than to the root ability to resist the entry of excess Fe^{++} into plants. This suggestion was supported by the experiments of Yamanouchi *et al.*[5], in which Fe^{++} was applied directly to detached leaves through cut end via transpiration stream.

Large inconsistency about genotype difference in tolerance for iron toxicity have been reported. The tolerance seems to vary with culture media (solution or soil), soil property, growth season, and nutritional status[3,6]. We reported that Fe^{++} stimulates ethylene production in detached rice leaves[7]. But the Fe^{++}-induced stress ethylene production (SEP) has not yet been understood in terms of plant age, leaf position, and genotype. In addition, no information is available on the relationship between SEP, bronzing, and tissue Fe concentration. Understanding the relationship may help to characterize those tolerant genotypes.

Since SEP is often concomitant with visible injuries, it has been suggested as an indicator of injury from a wide range of environmental stresses[8]. In practice, diagnosis during period of "invisible" injury is more necessary but difficult than diagnosis after visible injury. In studies on air pollution (e.g., SO_2 and O_3) damage, stress ethylene formation during invisible injury has been regarded as a measurement of latent lesions or cellular disturbance[9,10]. This paper aims to determine whether there is a relationship between SEP, tissue iron concentration, and bronzing development.

1 Materials and methods

1.1 Plant cultivation and treatments

Seeds were obtained from the International Rice Germplasm Center and the Plant Breeding, Genetics, and Biochemistry Division of the International Rice Research Institute.

Experiment 1: Twelve pregerminated seeds of each variety were sown in a circular row in a 4-liter plastic pot which contained 3.5kg of Maahas clay soil fertilized with 5g of complete fertilizer (14% N P K) and 10g of corn starch. The plants (genotype: IR5 and IR20) were

grown in a screenhouse (28－32 ℃ , natural light). Tillers were cut several times, leaving only the main culm to grow. At 40 days after sowing (DAS), 1g of ammonium sulfate was applied as topdressing. The first, second, and third leaf from the top were cut at the length of 20cm from the leaf tip at different stages during 30－84 DAS, and then the cut end of each leaf was dipped into 5ml of 3.52mmol/L $FeSO_4$ (pH 5.0) in a test tube (2.5×20cm), about 3cm of cut end was under the solution surface. Incubations were done in a growth chamber (28 ℃ , 70%RH, 12h 195.3μmol m^{-2} s^{-2} light/ 12h dark) using completely randomized design with 3 replications.

Experiment 2: 16 genotypes were grown, the second and third leaves were cut at 55 DAS and treated, each step was done in the same way as in experiment 1.

Experiment 3: The plants were grown in culture solution to study SEP in whole plants. Pregerminated seeds were sown on a sheet of nylon net stretching on a styrofoam plate (25×32cm) with 80-holes (1.5cm diameter). One seed was sown in each hole. The plate was held in a 7.5-liter plastic tray with complete nutrient solution (pH 5.0) according to Yoshida *et al.*[11]. The plants were grown in a phytotron glasshouse (27/21 ℃, day/night) for 3 weeks. The plants were then transferred to the culture solution containing 0 and 5.28mmol/L of Fe^{++} ($FeSO_4$) and grown in a growth chamber (27/21℃, 12h 390.6μmol $m^{-2}s^{-2}$ light/12h dark, 70%RH). The solution was renewed every day. The roots and shoots were separately sampled at various days after treatment for ethylene production and Fe content measurement.

Experiment 4: The plants were normaly grown for 3 or 4 weeks in a screenhouse (28－32 ℃, natural light) in the same way as in experiment 3, then partially or totally derooted by cutting roots at different length from the tip, placed in the test tubes (2.5×20cm) containing 40ml of 0 or 5.28mmol/L Fe^{++} in nutrient solution, and then incubated under room condition for 24h(25－28 ℃, ca. 10h room light per day). The second and third leaves from the top were detached (20cm from the tip) to measure ethylene production and Fe content. Total root length was measured by Comair root length scanner. The degree of root-cutting was expressed as percentage of cutting length out of the total root.

1.2 Measurement of ethylene production

Ethylene production of the plant was determined according to Peng and Yamauchi[7]. SEP (nL g^{-1} fresh weight h^{-1}) was expressed as:

C_2H_4 in treated tissues－C_2H_4 in untreated tissues.

1.3 Determination of Fe concentration

About 0.1g of dried (80 ℃, 3 days) and ground sample was predigested overnight with

10ml of concentrated nitric acid in a 125-ml flask. The predigested sample was then heated slowly to remove red fumes from the solution and cooled. Three ml of 60－62% perchloric acid was then added. The mixture was heated again until it became clear, and then cooled. The solution was then filtered and diluted to 100ml with deionized water. The Fe concentration was determined by atomic absorption spectrophotometry (Perkon Elmer 3100). ICI (μg Fe g^{-1} dry weight) was expressed as:

Fe in treated tissues－Fe in untreated tissues.

1.4 Scoring of bronzing intensity

The detached leaves were treated with 3.52mmol/L of Fe^{++} for 24h, then the SEP was determined. After ethylene measurement, the leaves were transferred to test tubes with distilled water and incubated at 30 ℃ in the dark for another 2 days, then bronzing was scored. Symptom scoring and stress ethylene determination were not made at the same time because 1-day treated leaves had no obvious symptoms other than a grey color scattered on the green background. Scoring was done using the International Rice Testing Program's scale for iron toxicity[11].

1.5 Statistical analysis

The plants in the phytotron and screenhouse and the treatments in the laboratory were laid out in completely randomized design with 3 replications. IRRISTAT was used for the correlation analysis.

2 Results

2.1 SEP in detached rice leaves and genotype differences

The SEP in detached leaves of two typical genotypes, IR5 (susceptible to the toxicity of iron) and IR20 (tolerant) was determined. The leaves of IR5 produced higher stress ethylene than those of IR20 when they were treated with 3.52mmol/L of $FeSO_4$. Especially for the second and third leaves from the top, the genotypic difference is very significant throughout the growing period (Fig. 1 A, B, C). This result suggested that the SEP might reflect the toxicity intensity. Unexpectedly, the ICI in the leaves (Fig. 1 D, E, F) was not associated with the SEP.

The hypothesis that SEP may measure leaf injury caused by Fe^{++} was further tested by using the 2nd and 3rd leaves of 16 genotypes at 55 DAS (Fig. 2). Significant correlation was obtained between the SEP and the bronzing ($r=0.659^{**}$). The ICI correlated with neither the SEP nor the bronzing.

2.2 SEP in whole plants and the role of roots

From the 16 tested genotypes we chose two genotypes that were even more typical than

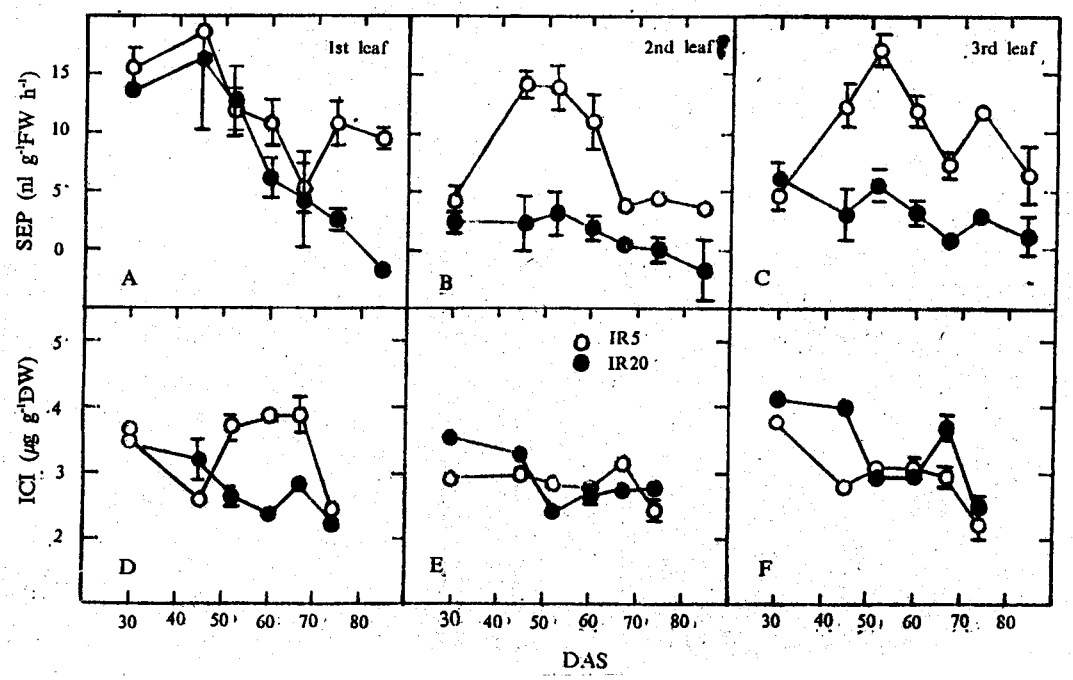
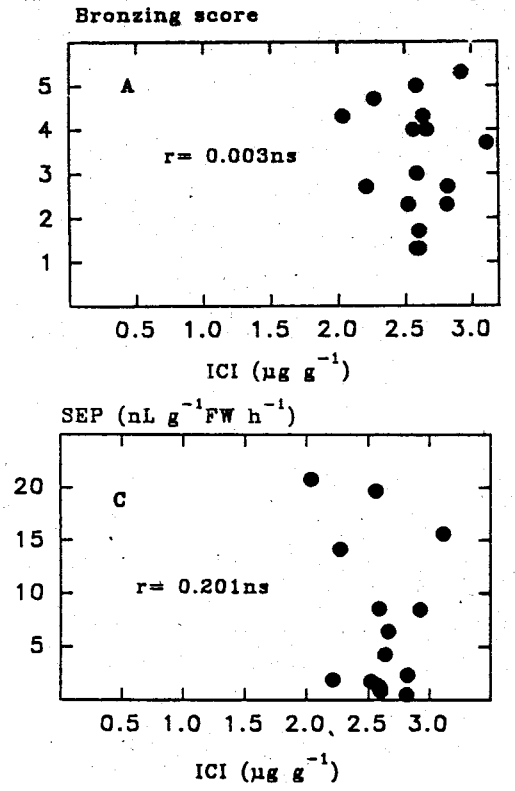


Fig. 1 Stress ethylene production (SEP, A, B, C) and iron concentration increment (ICI, D, E, F) in detached leaves from 2 rice genotypes. Different positional leaves were excised from the plants at indicated growth stages, then treated with 3.52mmol/L Fe^{++} for 24h (Bars represent standard errors).



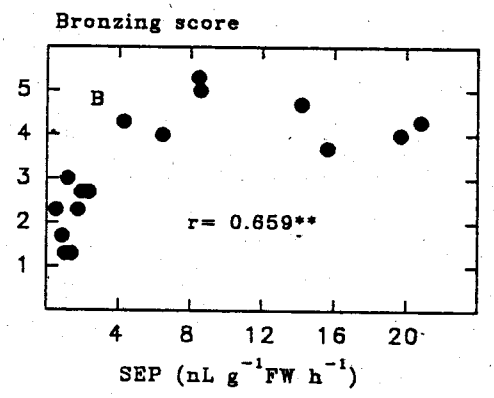


Fig. 2 Correlation between stress ethylene production (SEP), iron concentration increment (ICI), and bronzing appearance in 16 different rice genotypes. The 2nd and 3rd leaves were detached from 55-day-old plants, treated with 3.52mmol/L Fe^{++} for 24h, and then ethylene was measured. The treated leaves were then transferred to distilled water, incubated for another 2 days, and then scored for bronzing and Fe uptake.

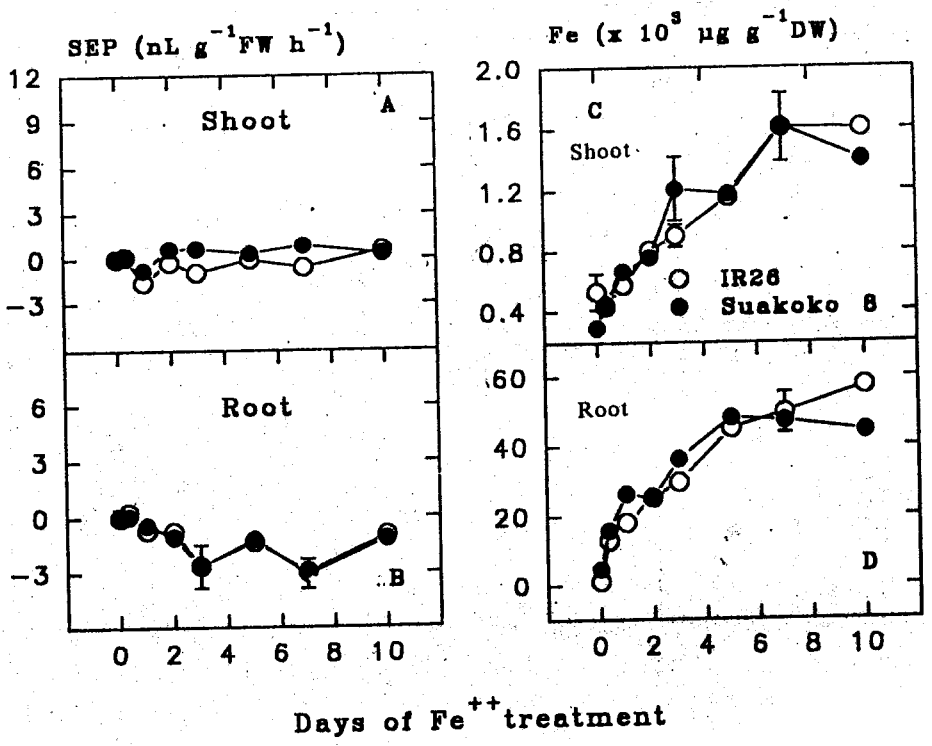


Fig. 3 Stress ethylene production (SEP) and iron accumulation in the whole rice plant. Three-week-old plants were treated with 5.28mmol/L Fe^{++} for 0–10 days (Bars represent standard errors).

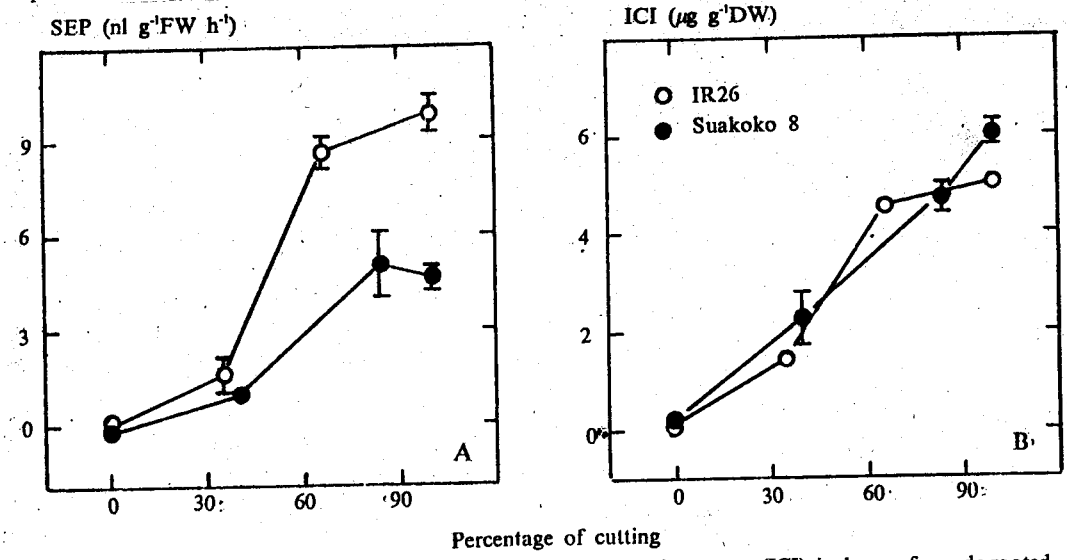


Fig. 4 Stress ethylene production (SEP) and iron concentration increment (ICI) in leaves from de-rooted rice plants. Three-week-old plants grown in culture solution were de-rooted at various degrees and treated with 5.28mmol/L Fe^{++} in nutrient solution for 24h. SEP and ICI in the second and third leaves were then determined (Bars represent standard errors).

IR5 and IR20 for the further study. IR26 was very sensitive and Suakoko 8 very tolerant

to iron toxicity as evaluated both by bronzing symptoms and SEP. Unexpectedly, in the whole plants of these two genotypes cultured in nutrient solution, ethylene production from the shoot was not much affected by the Fe^{++} treatment, it was even inhibited in the root (Fig. 3 A, B). Iron concentration increased to 1 600 μg g^{-1}DW in the shoots and to 40 000 μg g^{-1}DW in the roots 10 days after treatment. Little difference in the SEP, iron concentration, and bronzing intensity between IR26 and Suakoko 8 was identified. The SEP and bronzing development also showed little difference between the other tested genotypes such as IR5, IR20, IR8, IR42, IR46, Mashuri (data not shown).

When the plants were partially or entirely de-rooted, and then grown in the solution with excess Fe^{++}, stress ethylene could be evoked in the leaves. Iron concentration increased with increasing the percentage of root cutting. The leaf of IR26 produced more stress ethylene than that of Suakoko 8 did, although ICI was similar between the two genotypes (Fig. 4).

3 Discussion

Stress ethylene production was usually considered as a response of plants to adverse conditions. Stress ethylene can be evoked by free radicals such as O_2, OH, or ferryl radicals[12,13]. Those radicals also can be quickly elicited by the oxidation of Fe^{++} in plant tissues[14,15]. They may attack plant cells, i.e., damage membrane, nick DNA, inactivate enzymes and proteins, break up cellular integrity, etc.[1,16,17]. Stress ethylene has been suggested to be an indicator of extent of injury or a measurement of latent lesions or cellular disturbance in various environmental stresses, especially in SO_2 and O_3 pollution[9,18]. Our study indicated that there existed significant correlation between bronzing intensity and SEP by using the detached leaves of 16 genotypes (Fig. 2). The leaf tolerance may contribute to genotype difference. The difference may come from both apoplast-excluding capacity[19] and symplast mechanisms to cope with ferrous toxicity. Oxidation of ferrous without free radical production by phytoferritin might be one way for the symplast to detoxify ferrous effects[1], and the other way would be the free radical scavenging systems, e.g. superoxide dismutase, peroxidase, and catalase.

It must be noticed that the SEP from the detached leaves differed not only with genotype, but also with leaf position and growth stage (Fig. 1 A,B,C). A question was, thus, raised as to why the first leaf, which usually showed less bronzing symptom[5], produced similar or even more stress ethylene than the lower leaves, which showed heavier bronzing symptom as usual. One explanation is that bronzing symptoms may not reflect the *in situ* injury in this topmost elongating first leaf, or in some genotypes that failed to show leaf symptoms even though plant growth was already severely inhibited by Fe^{++}[4].

Bronzing symptom is thought to be caused by precipitation and deposition of ferric compounds[6,7]. Therefore, it should be noted that the location of the precipitation appears to be critical for the toxicity to the tissue[1,20]. When Fe^{++} uptake is slow, the cell wall and associated polysaccharides are supposed to have enough capacity to exclude Fe^{++} from the symplast[19]. Oxidization of Fe^{++} in the apoplast may also cause bronzing symptom, but intercellular injury might not occur because the elicited free radicals do not have enough time to enter the cell[1]. In this case bronzing symptoms may not relate to the injury of the tissue. This could also be the reason why the SEP from the solution-cultured whole plants was little influenced by the Fe^{++} treatment, here the rate of ICI was less than one-tenth of that in detached leaves (Fig. 1, Fig. 3). This notion was further confirmed by treating the de-rooted plants. When the root was partially or totally cut, resulting in an increased Fe^{++} uptake,, stress ethylene was evoked by Fe^{++} treatment (Fig. 4). Apparently, once the exclusion capacity of the apoplast is overcome due to a fast Fe^{++} accumulation, Fe^{++} may enter the symplast, then bronzing symptom, injury and SEP can occur in parallel pattern even though they may not be induced simultaneously. This appears to be the case in detached leaves and in de-rooted plants.

As long as rice plants are grown in well-nourished soil without root damage, roots keep Fe^{++} uptake at a slow rate. If roots are mechanically injured like by transplanting or toxic soil, excess Fe^{++} will enter the plant at a high accumulation rate. Then the SEP may depend on leaf tolerance. It has also been suggested that iron oxidation in the rhizospere may hinder the uptake of essential nutrients[21,22]. Moreover, the high Fe^{++} level in the rice roots (Fig. 3D) may impair the normal functions of the roots *per se*. Consequently, both plaque and perturbation of root function can retard nutrient uptake, indirectly affecting shoot growth[3,6]. Therefore, root function is also an important factor that accounts for rice to cope with iron toxicity.

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