

## Evaluating N-free Solution Culture System for Determination of Nitrogen Fixation in Rice

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### ABSTRACT

*Nitrogen fixation by endophytic bacteria has a potential to contribute to the mitigation of global warming by reducing the use of nitrogen fertilizer and thus emission of nitrogen oxide. Screening rice and associated nitrogen fixing endophytic bacteria is limited by a lack of appropriate N-free nutrient solution. This study set out to evaluate N-free nutrient solutions and systems for growing rice, by evaluating nutrient solution with 2 different forms of iron (Fe), Fe-citrate and Fe-EDTA for rice growth and N levels (0 and 70 ppm,  $N_0$  and  $N_{70}$ ). Five rice seedlings were grown in each pot containing 5 L of either nutrient solution with 2 replications. In one set of pots the nutrient solution was not renewed and in another set the nutrient solution was renewed weekly. It was found that chlorophyll content (measured with SPAD502 on the youngest emerged blade) varied with N levels and form of Fe. The SPAD value of  $N_{70}$  was higher than  $N_0$  in both iron forms. Nitrogen deficiency in  $N_0$  was indicated by chlorosis and necrosis of older leaves and greener younger leaves. In  $N_0$  without nutrient solution renewal, the SPAD value was higher with Fe-EDTA than Fe-citrate, with Fe deficiency of Fe-citrate plants indicated by more chlorosis of younger than older leaves. Rice plants were grown successfully in  $N_0$  with EDTA-Fe in enclosed root system without renewal for one month with 1 L, and longer with 1.5 and 6 L of nutrient solution. The system will now be used for evaluating nitrogen fixation in rice in association with endophytic bacteria.*

**Key words:** Rice, Nitrogen fixation

### INTRODUCTION

Rice (*Oryza sativa*) is an important agricultural crops in the world, feeding more than 50% of the world's population (Hossain and Fischer, 1995; Ladha et al., 1997; Gyaneshwar et al., 2001). According to the higher demand of rice consumption, its yields will need to be enhanced to match the consumption. To achieving these higher yields by 2020, it will require at least double the amount of N fertilizers currently being used. This is because after water, N is the most limiting nutrient for rice growth (IRRI, 1993). The use of nitrogen fertilizer is of great importance in rice production, as nitrogen is the major factor limiting growth under most conditions (Dawe, 2000). Nitrogen fertilizers derived from nonrenewable energy resources such as petroleum and natural gas to meet the demand for food grain, which may cause environmental pollution and global warming.

Since agriculture is expected to move toward environmentally sustainable methods (Sturz et al., 2000). An alternative to increase the use of chemical fertilizers is to explore and improve the ability of rice to obtain N from biological  $N_2$  fixation (BNF) (Ladha and Reddy, 1995; Wu et al., 1995). Biological N fixation by endophytic bacteria has the advantages of lower cost and reduced environmental hazards and is more consistent with the development of sustainable agriculture. Beside, endophytic nitrogen fixing bacteria can contribute to global warming mitigation. However, screening rice and associated nitrogen fixing endophytic bacteria is limited by a lack of appropriate N-free nutrient solution. This study aimed to evaluate the N-free nutrient solutions and systems for growing rice by evaluating iron form and N level in nutrient solution and volume of nutrient solution.

## MATERIALS AND METHODS

### Evaluation of nutrient solution with different forms of iron (Fe) and nitrogen (N) levels

A rice genotype (Chai Nat 1, CN1) was grown in N-free nutrient solution varied by forms of Fe, levels of N and frequency of renewal, based on the formula used by Mc Donald et al. (2001) and Insalud (2006). The 2 different forms of Fe were Fe-citrate and Fe-EDTA, with 2 N levels (0 and 70 ppm,  $N_0$  and  $N_{70}$ ). The nutrient solution was renewed weekly or not at all for the entire experimental period. The seeds were placed on a plastic net in plastic pot until germinated. Ten days after germination, five plants were transplanted to plastic pots containing 5 L of nutrient solution. The experiment was conducted with independent duplicates of the factorial treatments.

### Evaluation of growth of rice plants in enclosed root system

Results from the first study led to the selection of the Fe-EDTA as the non-renewable N-free nutrient solution to evaluate the growth of rice in enclosed root system. Seedlings with 3 leaves were transplanted individually in each plastic bottle containing 1, 1.5 or 6 L of the nutrient solution, in triplicate. The plants were grown without the nutrient solution being changed or modified in any way for one month.

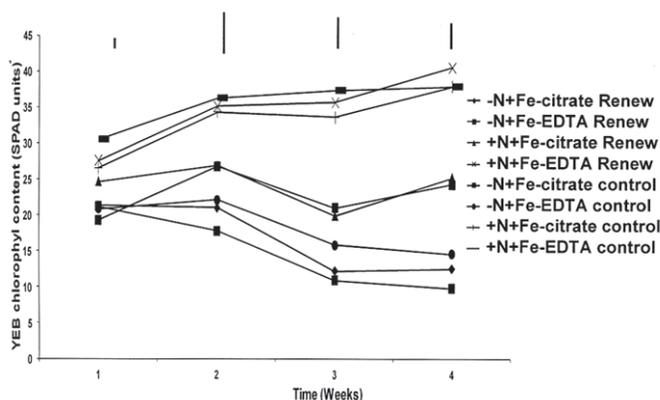
### Data collection

Data recorded were chlorophyll content (SPAD 502) in YEB (youngest emerged blade) weekly after transplanting, number of leaves plant<sup>-1</sup> and tillers plant<sup>-1</sup> at 4 weeks after transplanting. Then, dry weight of shoot and roots (g plant<sup>-1</sup>) were measured.

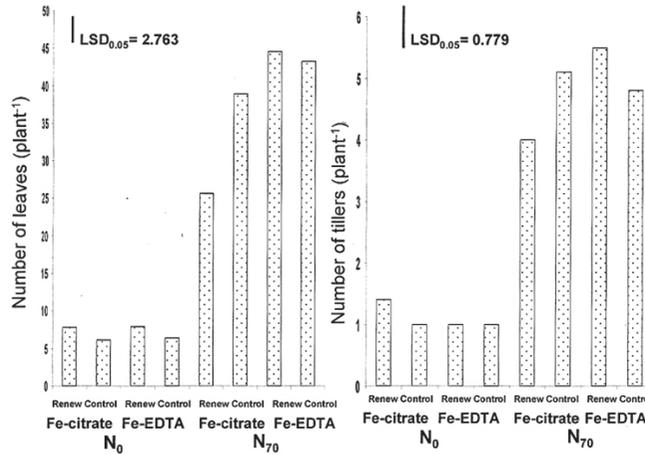
## RESULTS

### The nutrient solution with different forms of iron and levels of nitrogen

Nitrogen and iron (Fe) deficiency of the rice plants were indicated by the YEB chlorophyll content, which varied with N levels and Fe forms. The SPAD value of  $N_{70}$  was higher than  $N_0$  in both iron forms (Figure 1). In  $N_0$  without nutrient solution renewal, the SPAD value was higher with Fe-EDTA than Fe-citrate. Besides, there was higher number of leaves and tillers in  $N_{70}$  than in  $N_0$  at 4 weeks after transplanting (Figure 2). In  $N_{70}$  with Fe-EDTA, number of leaves was significantly higher than that of Fe-citrate in all nutrient solution with and without renewal. The number of tillers in  $N_{70}$  Fe-citrate with nutrient solution renewal declined over time, whereas Fe-citrate without nutrient solution renewal was similar when compared to Fe-EDTA with and without nutrient solution renewal. In  $N_0$ , number of leaves and tillers was not significantly different in all Fe forms.

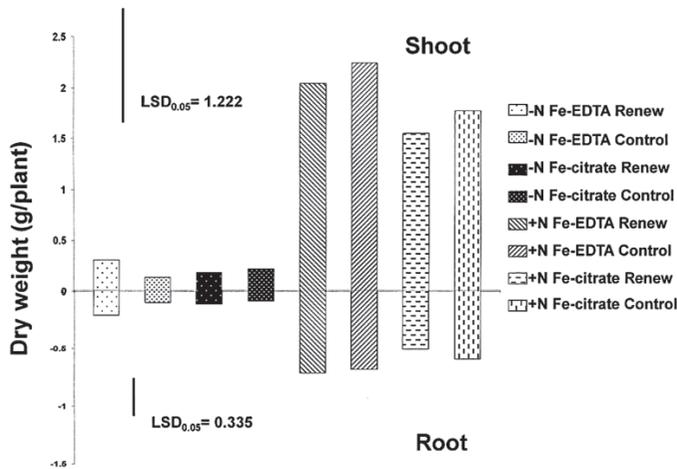


**Figure 1.** YEB chlorophyll content of rice grown in 2 N levels ( $N_0$  and  $N_{70}$ ) and iron forms (Fe-EDTA and Fe-citrate) without nutrient solution renewal and weekly renewal.



**Figure 2.** Number of leaves and tillers (plant<sup>-1</sup>) of rice grown in 2 N levels (N<sub>0</sub> and N<sub>70</sub>) and iron forms (Fe-EDTA and Fe-citrate) without nutrient solution renewal and weekly renewal.

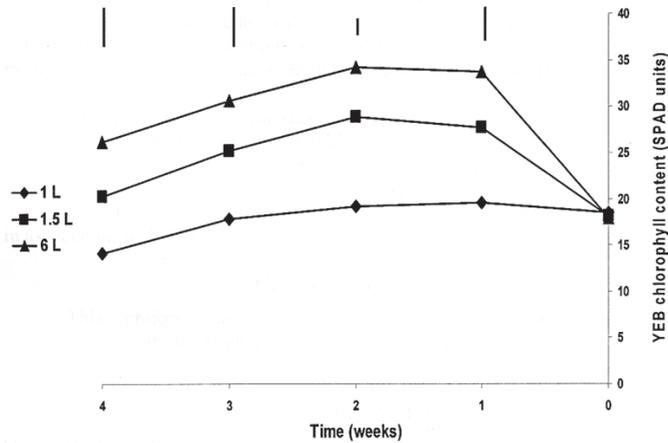
The effect of N deficiency in N<sub>0</sub> on shoot dry weight and root dry weight were clearly observed at 4 weeks after transplanting, but there was no difference between the Fe forms in both N levels (Figure 3). In nutrient solution renewal, root dry weights did not differ significantly between the Fe-EDTA in N<sub>0</sub> and Fe-citrate in N<sub>70</sub>.



**Figure 3.** Shoot and root dry weight of rice grown in 2 N levels (N<sub>0</sub> and N<sub>70</sub>) and iron forms (Fe-EDTA and Fe-citrate) without nutrient solution renewal and weekly renewal.

**Evaluation of growth of rice plants in enclosed root system**

CN1 grown in container with different nutrient solution volume (1, 1.5 and 6 L) were significantly different in their YEB chlorophyll content at all containers (Figure 4). The YEB chlorophyll content in 6 L container was higher than 1.5 and 1 L, respectively. The number of leaves did not differ significantly between the containers at 4 weeks after transplanted (Table 1).



**Figure 4.** YEB chlorophyll content of rice grown in  $N_0$  with Fe-EDTA without nutrient solution renewal for one month.

**Table 1.** Number of leaves (plant<sup>-1</sup>) of rice grown in  $N_0$  with Fe-EDTA without nutrient solution renewal for one month.

Volume of containers	Number of leaves
1 L	5.6
1.5 L	6.0
6 L	6.6
Mean	6.1 <sup>ns</sup>

<sup>ns</sup> Not significant

## DISCUSSION AND CONCLUSION

The present result clearly showed that the SPAD value of  $N_{70}$  was higher than  $N_0$  in both iron forms. This evidence confirmed the previous investigation that nitrogen and iron deficiencies in the rice plants can be indicated by the YEB chlorophyll content, because nitrogen is an essential constituent of chlorophyll. Nitrogen deficiency symptom in rice is become light green in old leaves and sometimes all leaves (Dobermann and Fairhurst, 2000). Beside, this study demonstrates that number of tiller, leave, shoot dry weight and root dry weight were reduced when grown in nitrogen deficiency. This is similar with the previous phenomenon that nitrogen deficiency resulted in reducing tillering and crop biomass production which was related to the rate of leaf photosynthesis (Dobermann and Fairhurst, 2000).

The SPAD value was higher with Fe-EDTA than Fe-citrate in  $N_0$  without nutrient solution renewal. This indicated the relationship of the deficiency symptom and Fe form which is seem to be more deficiency symptoms with Fe-citrate than Fe-EDTA. This is probably due to the higher availability of Fe-EDTA than Fe-citrate (Marchner, 1995). The symptom of Fe deficiency from Fe-citrate indicated by the more chlorosis of younger than older leaves (Terry and Abadia, 1986; Dobermann and Fairhurst, 2000).

Rice plants were grown successfully in  $N_0$  with Fe-EDTA in enclosed root system without renewal for a month with the total volume of 1 L, and longer with 1.5 and 6 L of nutrient solution. The present results suggest that the appropriate nutrient solution for rice growth should be used Fe-EDTA and contained the nutrient solution of 1.5 L/month. The system will now be used for evaluating nitrogen fixation in rice in association with endophytic bacteria.

### ACKNOWLEDGEMENTS

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