Using Agar Nutrient Solution to Study Rice Zinc Deficiency

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INTRODUCTION

Zinc deficiency is a widespread micronutrient disorder in lowland rice that has been associated with a wide range of soil conditions: low available Zn, low redox potential, high pH, high bicarbonate content and prolonged submergence (Neue and Lantin 1994). These compounding factors make it difficult to reproduce Zn-deficiency symptoms as found in the field when growing rice in nutrient solution. It is particularly difficult to maintain high pH, and to prevent Zn contamination of the culture medium from the surrounding environment in conventional nutrient solution culture. Recently, chelator-buffered nutrient solutions have been used for studying Zn deficiency in plants, since it is easier to induce Zn deficiency in them than in conventional nutrient solutions. However, chelators used for buffering micronutrient activities are usually large synthetic compounds that potentially have adverse effects on plants (Rengel 1999). For example, rice seedlings suffered severe Fe deficiency when growing in DTPA buffered nutrient solution (Yang et al. 1994). Therefore, a new technique is required for studying Zn deficiency in rice. In this experiment, an agar nutrient solution was used to mimic Zn-deficient conditions as seen in rice fields. Some problems that occur in conventional or chelator-buffered nutrient solutions can be solved by using agar nutrient solution.

METHODS

Agar was dissolved in boiling deionized water, and then mixed with Yoshida nutrient solution. Potassium hydrogen carbonate ($KHCO_3$) was added to maintain high pH. The final agar concentration was 0.1% (w/v). Plants were supplied with a low (0.1×10^{-3} μM) or sufficient (1.5μM $ZnSO_4$) Zn treatment. Four rice genotypes with contrasting Zn-deficiency tolerance were selected based on field screening studies and tested in the agar nutrient solution. Lines 46 and 507 were identified as Zn efficient, whereas IR74 and M11 were Zn-inefficient genotypes. Pre-treated (two weeks without Zn) rice seedlings with four leaves were transplanted into agar nutrient solution or one strength Yoshida solution with different Zn treatments. The pH and redox potential of agar and conventional nutrient solution were monitored throughout the experiment. Plants were harvested after Zn-deficiency symptoms fully developed. Leaf bronzing was scored based on the classification by Wissuwa (2006). Shoots and roots were separated, oven dried at 70^{o}C for 3 days, and dry weights were recorded.

RESULTS AND DISCUSSION

Plant growth reduction and Zn deficiency symptoms

Plants with low Zn supply showed stunted growth, such as reduction in plant height, lower leaf numbers and smaller leaf blades, compared to plants with a sufficient Zn treatment. In general, growth reduction caused by Zn deficiency was more severe in agar than in conventional nutrient solution (Table 1).

After one week of growth in agar nutrient solution, Zn-inefficient rice genotypes IR74 and M11 displayed Zn-deficiency symptoms. Reddish-brown spots on upper leaves of stunted plants developed into reddish-brown blotches and streaks, which then fused to cover leaves entirely. Leaves then lost turgor, turned brown and finally dried up. The Zn-inefficient genotypes grown in conventional nutrient solution showed visual Zn-deficiency symptoms seven days after those grown in agar, indicating that agar nutrient solution is a more effective medium for inducing Zn deficiency. The bronzing scores of plants grown in conventional nutrient solution were lower than that of plants grown in agar nutrient solution, indicating that Zn deficiency is more severe in agar nutrient solution (Table 1). No visual Zn deficiency symptoms were observed in Zn-efficient genotypes 46 and 507 throughout the experiment.

Table 1. Zinc deficiency symptoms of rice seedlings grown in agar and conventional nutrient solution at a low level of Zn (0.1×10^{-3} μM) and their relative performance compared to sufficient Zn (1.5 μM) supply.

Genotype	Treatment			
	Agar nutrient solution		Conventional nutrient solution	
	Relative dry matter	Leaf Bronzing	Relative dry matter	Leaf Bronzing
	(%)	(Score)	(%)	(Score)
46	65	0	73	0
507	44	0	57	0
IR74	44	7	44	4
M11	47	8	60	7

pH and redox potential of the culture medium

The medium pH decreased one unit in seven days in the agar nutrient solution, redox potential (Eh) declined by approximately 300 mV. In contrast, the redox potential of conventional nutrient solution remained stable, while pH decreased dramatically by 2.5 units in seven days.

Table 2. Culture medium pH and Eh changes of agar and conventional nutrient solution.

Zn supply	Treatment							
	Agar nutrient solution				Conventional nutrient solution			
	pH		Eh(mV)		pH		Eh(mV)	
	Day1	Day7	Day1	Day7	Day1	Day7	Day1	Day7
0.1×10^{-3}μM	7.0	6.0	293	-56	7.0	4.5	484	500
1.5μM	7.0	6.0	313	-14	7.0	4.5	496	501

CONCLUSIONS

The use of agar nutrient solution is efficient to study Zn deficiency in rice seedlings. Using agar nutrient solution induces Zn deficiency more rapidly than conventional nutrient solution without the addition of chelators because of the lack of convection in 0.1% agar nutrient solution (Wiengweera et al. 1997). This is preferable for the formation of a Zn-depleted rhizosphere, mimicking the situation occurring in a paddy soil. In addition, the stagnant agar solution was anaerobic and chemically reduced, which is usually the situation in paddy soil.

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