



The Effectiveness of Nutrient Culture Solutions with Agar Addition as An Evaluation Media of Rice Under Iron Toxicity Conditions

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ABSTRACT

Background: Evaluation of the tolerance level of rice to iron (Fe) toxicity stress can be done using a hydroponic system in a nutrient culture solution under a controlled condition. This study aimed to obtain a nutrient culture solution that effective as a medium for evaluating the response of rice under Fe toxicity stress condition. **Methods:** This experiment was carried out by comparing the effectiveness of three kinds of nutrient culture media, namely Yoshida's Half-Strength solution (HSY), Yoshida's Half-Strength + 0.2% agar solution (HSYA), and Yoshida's Full-Strength + 0.2% agar solution (FSYA) using two rice genotypes, Inpara 5 (sensitive to Fe toxicity) and Mahsuri (tolerant to Fe toxicity). Leaf bronzing level, plant dry weight, and pH of nutrient culture media were observed in this experiment. **Results:** The results showed that the stress response as represented by bronzing score in Inpara 5 leaves was known to be higher than that of Mahsuri in the three nutrient culture media. The decrease of root and shoot dry weight in Inpara 5 was higher than that of Mahsuri. In addition, the decrease in the pH of nutrient culture solution media without an agar addition (HSY) occurred faster than the media with the agar addition (HSYA and FSYA). **Conclusion:** The HSYA and FSYA media exhibited similar pattern of pH declining but causing significant different in growth responses between Inpara 5 and Mashuri indicating the HSYA medium is considered more efficient compared to the FSYA medium because it only requires a smaller amount of agar.

Efektivitas Larutan Kultur Unsur Hara dengan Penambahan Agar Sebagai Media Evaluasi Padi Dalam Kondisi Toksisitas Besi

ABSTRAK

Background: Evaluasi tingkat toleransi tanaman padi terhadap keracunan besi (Fe) dapat dilakukan menggunakan sistem hidroponik pada larutan kultur hara yang kondisinya terkontrol. Penelitian ini bertujuan untuk mendapatkan larutan kultur hara yang efektif sebagai media evaluasi respon tanaman padi pada kondisi cekaman keracunan Fe. **Metode:** Percobaan ini dilakukan dengan membandingkan efektivitas dari tiga macam larutan kultur hara, yaitu Yoshida's *Half-Strength* (HSY), Yoshida's *Half-Strength* + agar 0.2% (HSYA), dan Yoshida's *Full-Strength* + agar 0.2% (FSYA) dengan menggunakan genotipe padi Inpara 5 (sensitif keracunan Fe) dan Mahsuri (toleran keracunan Fe). Tingkat *bronzing* daun, bobot kering tanaman, dan pH media kultur hara diamati pada percobaan ini. **Hasil:** Hasil penelitian menunjukkan bahwa respon stres berupa *bronzing* daun Inpara 5 diketahui lebih tinggi dibandingkan Mahsuri pada ketiga macam larutan kultur hara. Penurunan bobot kering akar dan tajuk Inpara 5 terjadi lebih besar dibandingkan Mahsuri. Selain itu, Penurunan pH larutan media kultur hara tanpa penambahan agar (HSY) terjadi lebih cepat dibandingkan media dengan penambahan agar (HSYA dan FSYA). **Kesimpulan:** Tidak adanya perbedaan pola penurunan pH larutan antara HSYA dan FSYA serta adanya perbedaan respon yang signifikan antara tanaman padi genotipe Inpara 5 dan Mahsuri pada media kultur hara. Media HSYA dinilai lebih efisien dijadikan sebagai media evaluasi tanaman padi pada kondisi keracunan besi karena hanya memerlukan jumlah agar yang lebih sedikit dibandingkan FSYA.

Kata kunci:

Bronzing daun

Larutan agar

Padi



Introduction

Iron (Fe) is one of the micronutrients that play a critical role in an organism's growth and development. The element iron is involved in the processes of photosynthesis, respiration, and as a cofactor of various enzymes (Brumbarova & Bauer, 2008; Marschner, 1995). Since excess Fe can cause toxic conditions, a strategy is needed to regulate the amount of Fe present in cells. The critical limit for Fe concentration causing toxicity stress in plants is 300 ppm (Yoshida et al., 1976). Fe toxicity is a symptom associated with elevated Fe levels in cells and is detrimental to plants due to the oxidative stress it (Devi et al., 2016; Jeong & Connolly, 2009; Kampfenkel et al., 1995; Khabaz-Saberi et al., 2010; Zheng, 2010). Fe toxicity stress has been reported to inhibit root and shoot growth, induce the formation of Fe-plaque on the root surface, and reduce photosynthetic and transpiration activities (Dhiman et al., 2016; Elec et al., 2013; Kabir et al., 2016; Muller et al., 2017; Quinet et al., 2012). There are three hypotheses proposed related to the plant's tolerance mechanism to Fe toxicity stress: increasing the ability of root oxidation in the rhizosphere to oxidize Fe²⁺ to Fe³⁺, accumulating Fe in cells through compartment strategies, and detoxifying reactive oxygen species (ROS) through enzymatic and non-enzymatic reactions (Becker & Asch, 2005; Engel et al., 2012; Wu et al., 2016). However, the level of tolerance to Fe toxicity in each plant depends on its genotype, age, and environmental conditions (Engel, 2009).

Attempts to develop tolerant rice varieties require a thorough strategy, which includes a breeding to obtain tolerant rice to Fe toxicity (Matthus et al., 2015). Therefore, the strategy of screening technique is needed to support the attempt. Successful breeding efforts require reproducible, effective, and inexpensive screening techniques to identify any variations in a plant's stress response (Nugraha et al., 2015), including Fe toxicity. One of the hydroponic screening strategies that is considered effective to support plant breeding is the addition of agar to nutrient culture solution media (Asch et al., 2005; Nugraha et al., 2015; Wang et al., 2008). The addition of agar is intended to maintain the pH of the nutrient culture solution. The availability of nutrients for an organism is greatly influenced by the pH of the growing environment. In addition, nutrient culture solution using only half the concentration of nutrient content is also considered effective for rice screening, especially in iron toxicity (Suryadi, 2012). This study aimed to obtain a nutrient culture solution that is effective as a medium for evaluating the rice plant responses to Fe toxicity stress conditions. The results of this study are useful to support rice breeding strategy to obtain rice lines that are tolerant to Fe toxicity.

Methods

The research has been conducted in the Laboratory of Plant Physiology and Molecular Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, in October 2016 – February 2017.

Experimental Design

The experiment compared the effectiveness of three different nutrient culture solutions, namely the Yoshida's Half-Strength solution (HSY), Yoshida's Half-Strength + 0.2% agar solution (HSYA), and Yoshida's Full-Strength + 0.2% agar solution (FSYA) as evaluation media for Fe toxicity in rice. In this experiment, two rice genotypes were used, i.e., Mahsuri (tolerant to Fe toxicity stress) and Inpara 5 (sensitive to Fe toxicity stress).

The seed surfaces were sterilized using 1% (v/v) Sodium Hypochlorite (NaOCl) for 15 minutes and rinsed using distilled water. The seeds were then germinated in an incubator (27 °C) for 3 days. The uniform seedlings were then transferred to a perforated styrofoam board and floated on a 9 L (35 x 28.5 x 12) cm³ container. The containers were previously filled with 8 L of HSY solution (Yoshida et al., 1976) as a pre-culture solution for 2 weeks with the nutrient solution being replaced weekly with the same composition. The 2-weeks-old rice were then planted in 800 mL plastic pots (Ø: 8.5 cm; height: 15 cm) that had been filled with 750 mL of different nutrient solution. The nutrient solution in each pot experiment used three different solutions, namely HSY, HSYA, and FSYA. The HSYA and FSYA nutrient solutions were prepared by cooking 60 g of agar in 8 L of aquadest. Furthermore, the agar solution medium was cooled to a temperature of 60 °C and then added to a container containing 22 L of Yoshida's nutrient culture solution until it reached a final volume of 30 L with an agar concentration of 0.2% (w/v) for 40 pots. The type of agar used in this study was pure agar strength of 900.

Determination of Leaf Bronzing Score

The Fe toxicity level was determined by observing the leaf bronzing conditions and conducting a scoring. Determination of the bronzing score of each leaf was adapted from Shimizu et al. (2005) but only on the second, third, and fourth leaf profiles. The explanation for using three leaves in scoring was that the rice plant used in observation only had four completely opened leaves (vegetative phase/24-days old rice), but the oldest leaves were omitted to minimize the bias on the scoring. The bronzing profile observed at each leaf position was classified into five categories: leaves without bronzing (N), leaves that change color only at the tip and are estimated to be <10% (T), leaves that change color between 20% and

<50% (P), leaves that change color of 50-100% (W), and leaves that are rolled/dried/dead (R) (Figure 1). After combining the three leaf profile groups, the leaf bronzing score (LBS) was calculated on a scale of 1-7 using the provisions shown in Table 1.

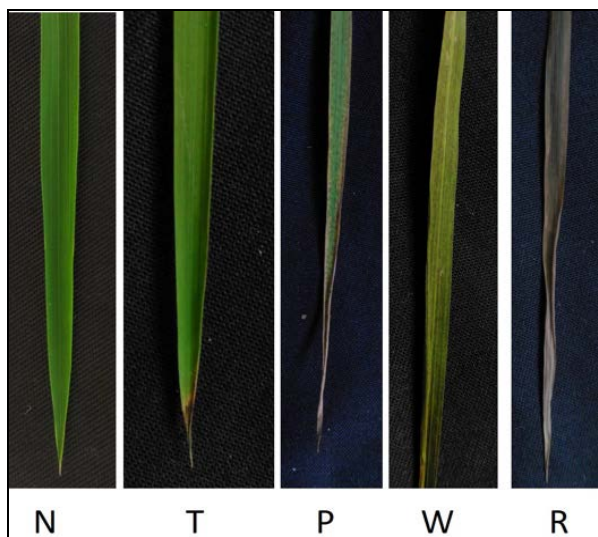


Figure 1. Categorization of leaf bronzing profiles in rice plant under Fe toxicity stress.

Table 1. Leaf bronzing score (LBS) levels

Skor	Leaf order		
	2 nd	3 rd	4 th
1	N	N	N
2	T	N	N
3	T	T	N
4	T/P	T	T
5	P	T/P	T
6	W	P	T/P
7	R	W/R	P/W/R

Note: N = leaves without bronzing; T = leaves that change color only at the tip and are estimated to be <10%; P = leaves that change color between 20% and <50%; W = leaves that change color of 50-100%; R = leaves that are rolled/dried/dead.

Observation of Plant Growth

After observing the leaf bronzing level on 10 days after treatment, the shoots and roots were harvested and then incubated in the oven at 70 °C for 72 hours to obtain dry weight data. Furthermore, dry weight data was used to determine the plant growth rate per unit time (per day).

Determination of the pH of Nutrient Culture Solution

The pH measurement of nutrient culture media was conducted on 0 day after treatment (seedlings before transplanting) then continued every day on the 1st to 10th days after treatment using a pH meter.

Statistical Analysis

The data obtained was subjected to analysis of variance at the 5% significance level using the SPSS 16.0 program.

Results and Discussion

The leaf bronzing score (LBS) and pH measurement were performed to compare the effectiveness of three different nutrient culture solutions as an evaluation medium in the Fe toxicity experiment. The LBS increased from 1st to 10th following Fe toxicity stress treatment (Table 2). The stress response shown by the sensitive genotype was higher than the tolerant genotype found in all three nutrient culture solutions. The results showed a significant difference (p<0.05) between the two genotypes since day 3 after Fe stress treatment (LBS3).

Table 2. Bronzing score (LBS) of rice leaves on 400 ppm Fe stress treatment during 10 days and different nutrient culture solution media

	Leaf Bronzing Score at day-										
	0	1	2	3	4	5	6	7	8	9	10
Genotype											
Inpara 5	1,0 ^a	1,1 ^a	1,6 ^a	2,0 ^b	3,3 ^b	4,0 ^b	5,5 ^b	7,1 ^b	7,5 ^b	8,1 ^b	8,5 ^b
Mahsuri	1,0 ^a	1,1 ^a	1,2 ^a	1,3 ^a	1,4 ^a	1,5 ^a	1,7 ^a	2,6 ^a	3,6 ^a	3,6 ^a	4,3 ^a
Nutrient culture solution											
HSY	1,0 ^a	1,1 ^a	1,4 ^{ab}	1,8 ^a	2,7 ^a	3,0 ^a	4,2 ^a	4,7 ^a	5,3 ^a	5,9 ^a	6,6 ^a
HSYA	1,0 ^a	1,2 ^a	1,8 ^b	1,8 ^b	2,5 ^a	2,9 ^a	3,6 ^a	5,0 ^a	5,7 ^a	5,9 ^a	6,4 ^a
FSYA	1,0 ^a	1,0 ^a	1,0 ^a	1,3 ^a	1,9 ^a	2,4 ^a	3,0 ^a	4,9 ^a	5,8 ^a	5,7 ^a	6,1 ^a

Note: N = leaves without bronzing; T = leaves that change color only at the tip and are estimated to be <10%; P = leaves that change color between 20% and <50%; W = leaves that change color of 50-100%; R = leaves that are rolled/dried/dead. Same letters in same column and factor means non-significantly different based Duncan's Multiple Range Test (DMRT) (α = 0.05).

In this study, the sensitive (Inpara 5) and tolerant (Mahsuri) genotypes were used following Nugraha et al. 2015 who also previously used both rice genotypes to study the effectiveness of nutrient culture solutions as evaluation medium for Fe toxicity stress in rice. However, in this study only used full-strength Yoshida's solution for evaluating the three types of solution media. In addition, the reason why the half-strength of nutrient solution was used in this study is because it is considered an effective concentration for rice screening against Fe toxicity stress (Suryadi, 2012). To maintain the solution pH stability, 0.2% of agar was added to the nutrient solution following previous studies (Nugraha et al., 2015).

Besides observing the plants' stress level based on the LBS value this study also revealed significant different (p<0.05) responses to different culture solution media between both rice genotypes as evidenced by their growth profiles (Figure 2). There was a significant decrease in root and shoot dry weight in both rice genotypes. The reduction in root and shoot dry weight in the sensitive genotype was greater than in the tolerant genotype (Figure 3a-b). Rice var. Inpara 5 in HSY culture solution demonstrated a 42.2 and 41.2 % decrease in root and shoot dry weight, respectively.

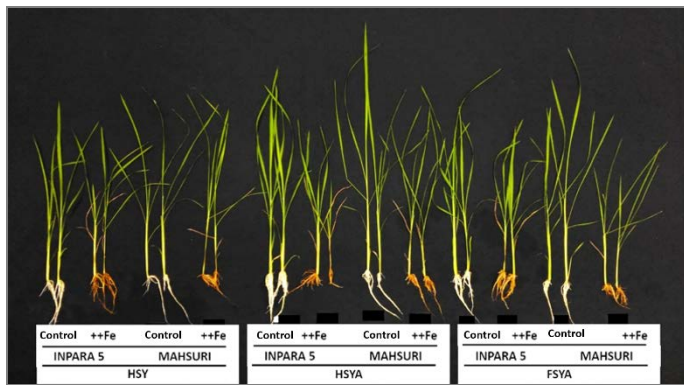
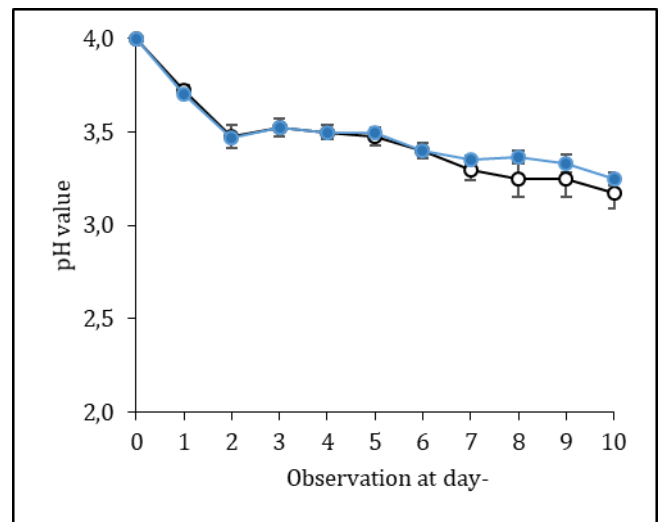
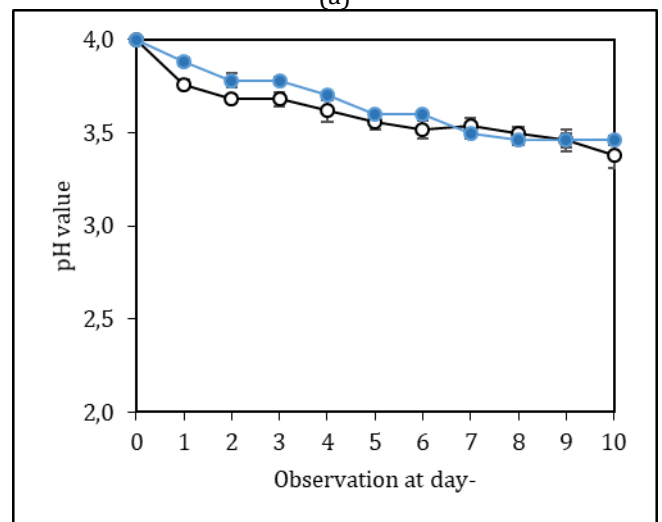


Figure 2. Growth profile of rice in three nutrient culture solutions. Control = 0 ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; ++Fe = 400 ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. HSY = Yoshida's Half-Strength solution; HSYA = Yoshida's Half-Strength + 0.2% agar solution; FSYA = Yoshida's Full-Strength + 0.2% agar solution.

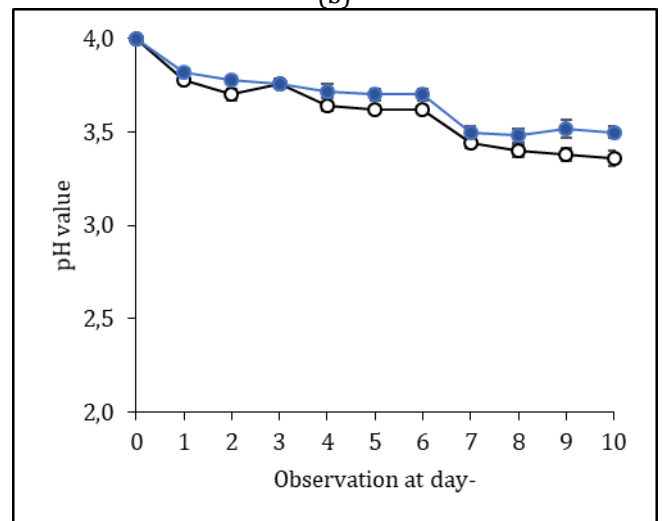
0.2% agar in nutrient culture solution causes inhibition of iron oxide (Fe_2O_3) precipitation.



(a)

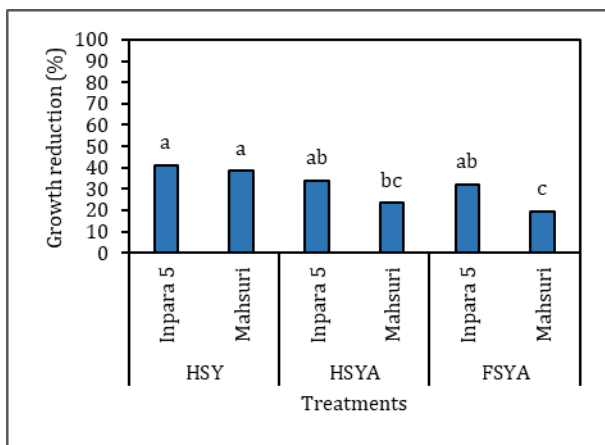


(b)

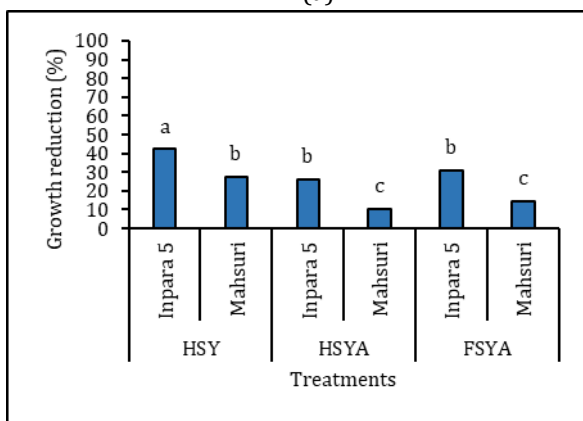


(c)

Figure 4. pH variation of HSY (a), HSYA (b), and FSYA (c) nutrient culture solution with 400 ppm Fe treatment during 10 days. HSY = Yoshida's Half-Strength solution; HSYA = Yoshida's Half-Strength + 0.2% agar solution; FSYA = Yoshida's Full-Strength + 0.2% agar solution; rice var. Inpara 5 (sensitive genotype to Fe toxicity) (—○—); rice var. Mahsuri (tolerant genotype to Fe toxicity) (—●—).



(a)



(b)

Figure 3. Reduction of shoot (a) and root (b) dry weight of Inpara 5 and mahsuri on three nutrient culture solutions. HSY = Yoshida's Half-Strength solution; HSYA = Yoshida's Half-Strength + 0.2% agar solution; FSYA = Yoshida's Full-Strength + 0.2% agar solution. Same letters above bar charts means non-significantly different based Duncan's Multiple Range Test (DMRT) ($\alpha = 0.05$).

The three different nutrient culture solution used this study also showed a significant difference ($P < 0.05$) in their pH changes (Figure 4a-c). In general, the addition of 0.2% agar to HSYA and FSYA nutrient culture solutions was able to slow down the decrease in pH compared to nutrient HSY culture solution without agar (Figure 4b-c). The use of

Therefore, the use of agar in nutrient culture solution was considered to be more effective as shown in several previous studies. A previous study conducted an addition of 1% agar to nutrient culture solution to study the response of several lowland rice genotypes to Fe toxicity stress (Asch et al., 2005). In addition, another study added 0.1% agar to study the rice responses to Zn deficiency and Fe stress (Wang et al., 2008). Based on the results of the study, the strategy of adding agar into the nutrient culture solution could be used in other nutrient studies involving other plant nutrients besides the Fe and Zn.

The addition of 0.2% agar in HSYA and FSYA solution was able to slow down the decrease in the solution pH when compared to HSY solution. The HSY solution demonstrated a significant decrease in the solution pH compared to HSYA and FSYA solution. The use of different nutrient culture solution affected the acidity level (pH) from 1st to the end of the experiment, which was the 10th days after Fe toxicity stress treatment. Our study also indicated that the pH of the HSY solution was consistently lower than the other two types of nutrient culture solution. The pH maintaining is a critical factor to consider when conducting Fe toxicity stress studies using the hydroponic method. This strategy aimed to maintain the uniformity of the Fe toxicity stress level in the solution because both pH of soil and solution greatly affect the availability of Fe for plants (Elec et al., 2013). The Fe concentration in the soil or reduced solution increased sharply with the decreasing pH. This was due to a decrease in the Fe²⁺ oxidation rate (Stumm & Lee, 1961).

The study found that the difference in nutrient culture solution had a significant effect on the rate at which the solution pH decreased. The solution pH in nutrient culture solution without agar addition decreased more rapidly compared to the solution with agar addition (Figure 3a). Hence, the addition of agar is essential to maintain the stable pH of the nutrient culture solution and maintain its effectiveness as a stress medium. The agar addition increased the viscosity and slowed the rate of oxygen diffusion into the solution, thus decreasing the rate of Fe-oxide precipitation (Li et al., 2016; Nugraha et al., 2015; Wang et al., 2008). Between the two types of nutrient culture solutions that used agar in this study, both HSYA and FSYA media showed relatively stable pH levels for 10 days of the 400 ppm Fe stress treatment.

This indicated that with the addition of 0.2% agar into the nutrient culture solution, it is not necessary to replace the solution daily. This strategy was consistent with the recommendations from previous study that the solution replacement in nutrient culture solution with the addition of agar can be achieved at intervals of 10-14 days (Wang et al., 2008). While there was no significant difference in pH stability of the solution, the HSYA solution was considered

more efficient because it requires less agar compared to FSYA solution.

Conclusion

Half Strength Yoshida nutrient culture solution with the addition of 0.2% agar (HSYA) was an effective and efficient media for evaluating the rice response to Fe toxicity stress.

Declaration statement

The authors reported no potential conflict of interest.

Acknowledgment

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