



AGRIVITA

Journal of Agricultural Science

Developments of Rice Cell Suspension Culture and A Novel Strategy for Screening New Resistant Lines to Rice Blight Disease Caused by *Xanthomonas oryzae* pv. *oryzae*

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ARTICLE INFO

Keywords:

Bacterial blight
Cell culture
Reactive oxygen species
Resistant cell lines
Screening

Article History:

Received: December 20, 2017

Accepted: August 13, 2018

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ABSTRACT

The research aimed to develop a rice cell culture system with high proliferation and screening resistant cell lines of rice to bacterial blight disease caused by *Xanthomonas oryza* pv. *oryzae* (Xoo). The culture cells obtained from the callus, cultured on CS-1 medium containing 3 % sucrose and 2 mg L⁻¹ 2,4-D for 4 weeks. The results showed that proliferation cell was significantly increased 1-fold in 3 weeks of primary culture in CS-1 conditioned medium (fresh/spent medium ratio 1:1) containing 3 % sucrose, 0.5 % glucose, 0.05 % fructose and 2 mg L⁻¹ 2,4-D. This medium was used to screen the cell lines through applying culture filtrate of Xoo. The method was to find a novel cell line which could produce high amounts of reactive oxygen species (ROS). Screening results showed 33 % cell lines were strong ROS-producing, two cell lines were selected and cultured for second round screening. The ratio of strong ROS-producing cell lines was increased up to 67 % in the third round screening. The strong ROS-producing cell lines in third round screening can be further cultured for plant regeneration. The rice cell lines with high ROS production may have potential of resistant cell lines against Xoo.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most widely cultivated crops in the world. Rice particularly important in Asia, where approximately 90 % of world's rice is produced and consumed. In most of developing countries, rapid population growth and economic development cause a constant increased food requirement such as rice demand. However, rice yield has been hovering in past decades, which is mainly caused by the absence of novel breeding technologies (Chen, Lin, & Zhang, 2009). In addition, the reduction of genetic diversity of rice cultivars may cause serious yield loss due to the increasing occurrences of insects, abiotic stresses and diseases. Disease caused by fungi, bacteria, viruses and nematode are important limiting factors on rice production (Song & Goodman, 2001).

Xanthomonas oryzae pv. *oryzae* (Xoo) is the causal agent of bacterial blight (BB) which causes

severe yield loss worldwide (Mew, Alvarez, Leach, & Swings, 1993). BB has also been spread to rice culture area worldwide, such as in Australia, Africa and Latin America as well as in North America (Ryba, Jean-Loup, & Leach, 1995). Moreover, BB has becomes a major rice disease in tropical Asian countries in last three decades due to the extensive deployment of more nitrogen-responsive rice varieties.

So far, the application of resistant varieties and systemic bactericides are the most widely used for controlling BB (Niño-Liu, Ronald, & Bogdanove, 2006). However, the application of bactericides has some limitations, such as not durable efficacy, existence of pathogenic variability, and chemical resistance strains derived in environment. Therefore, the alternative strategy to handle this disease is more considerable. One of the preferred strategy to control this disease is breeding resistant

ISSN: 0126-0537 Accredited by DIKTI Decree No: 60/E/KPT/2016

Cite this as: Kusuma, R. R., Sulistyowati, L., Cheng, C.-C., & Lin, Y.-H. (2018). Developments of rice cell suspension culture and a novel strategy for screening new resistant lines to rice blight disease caused by *Xanthomonas oryzae* pv. *oryzae*. *AGRIVITA Journal of Agricultural Science*, 40(3), 434–441. <http://doi.org/10.17503/agrivita.v40i3.1779>

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varieties which carrying resistance (R) genes. To date, 29 R genes to BB have been identified (Niño-Liu, Ronald, & Bogdanove, 2006). However, the consumer's concerns about the side effect of genetic engineering product and unstability of transgene has also limited this strategy for managing BB.

Tissue culture system has been known as a potential technique for crop improvement for several decades. In this technique, rice cell suspension culture with higher rate of somaclonal variation is a potential way to screen novel interested lines (Sharp, Evans, Ammirato, & Yamada, 1984). Therefore, this strategy has been done to screen disease resistant plants against different pathogens during past decades, such as potato lines against *Phytophthora infestans*, peach lines against *Xanthomonas* sp., and alfafa lines against *Fusarium oxysporum* on (Daub, 1986; Taji, Kumar, & Lakshmanan, 2002).

Cell suspension culture is performed by isolating single cells without removing the cell walls. Because the single cells with walls may regenerate their normal walls easier to form clonal colonies and embryos than those without walls (Hayashi *et al.*, 1994). Besides, it has been found that the growth, maintenance and regeneration of rice cell suspension were affected by several factors. These factors including basal medium, plant growth regulators, carbohydrate sources, nitrogen sources and varieties (genotype) (Hayashi *et al.*, 1994; Lee, Shultz, Hanley-Bowdoin, & Thompson, 2004). However, a suitable rice cell suspension system to maintain single cell often accompanied by slow rate of cell proliferation and death of the cells (Lee, Shultz, Hanley-Bowdoin, & Thompson, 2004). Therefore, a cell suspension culture system of rice with high cell proliferation need to be developed.

Screening resistant lines through enhancement of plant immunity system still undeveloped yet. In plant immunity system, the process of pathogen recognition by plant cell is an important part. This recognition is occurred by the reaction of pathogen-associated molecular patterns (PAMPs) and receptors on cell membrane of plant. While the reaction overpass the threshold of hypersensitive response (HR) at the infection site plants become disease resistant (Jones & Dangl, 2006). During this process, reactive oxygen species (ROS) is an important signal to lead program cell death and HR of plant cell. Therefore, cell lines with higher production of ROS may have potential to be resistant cell lines.

The objectives of this research was to develop a rice cell suspension system with high proliferation and screening resistant cell lines of rice (*Oryza sativa* var. japonica cv. Taiken 9) to bacterial blight disease caused by Xoo.

MATERIALS AND METHODS

The research was conducted in Laboratory of Plant Biotechnology and Laboratory of Bacteriology at National Pingtung University of Science and Technology (NPUST) Taiwan, from March 2012 - May 2013. The research was divided in two major parts including development of cell suspension culture system and development a screening system to select potential disease resistant lines against BB.

Callus Induction

The mature seeds of *Oryza sativa* var. japonica cv. Taiken 9 were collected from Laboratory of Plant Bioetchnology, NPUST and stored at 4 °C prior to use. The seeds were dehusked and washed by distilled water, then sterilized by 70 % alcohol, 5 % sodium hypochlorite (NaOCl), and rinsing 4 times with sterile distilled water. These dehusked seeds were cultured on CS-1 medium (Table 1) with 2 mg L⁻¹ 2,4-D, 3 % sucrose and 0.4 % gellan gum for 30 days.

Establishment Culture of Rice Suspension Cells

The suspension cells were generated from the active growing of dedifferentiated 30-days old callus. For about 1.0 g fresh weight of callus was used to establish cell suspension culture and put into 25 mL of CS-1 liquid medium as basal medium. The establishment culture of rice cell suspension were incubated on a rotary platform shaker (100-120 rpm) in room temperature. The cell number was determined after 2-weeks of culture.

Modification of Medium

Some parameters were tested including the plant growth regulator (PGR), carbohydrate source, vitamin and conditioned medium. The effects of PGR were determined by applying three concentrations of 2,4-D, 2 mg L⁻¹, 4 mg L⁻¹ and 6 mg L⁻¹. The effect of carbohydrate source on proliferation rate of rice cell suspension in primary culture was determined by three different concentrations of sucrose, glucose and fructose. The effect of thiamine addition was investigated to cell proliferation, 0.5 mg L⁻¹ thiamine, 2.5 mg L⁻¹ thiamine and 5 mg L⁻¹ thiamine. The

number of cell was measured each week. The best result of proliferation cells were continued for further experiment.

Four medium ratio was investigated on the proliferation of rice cell suspension culture in primary culture. The first medium was 1:1 ratio which means 50 % of fresh medium and 50 % previous medium, the second medium was 1:2 ratio which means 33.33 % of fresh medium and 66.66 % of previous medium, the third medium was 1:3 ratio which means 25 % of fresh medium and 75 % of previous medium, and the last medium was 1:0 ratio, it means 100 % of fresh medium.

Culture Filtrate of *Xanthomonas oryzae* pv. *oryzae*

X. oryzae pv. *oryzae* used in this study was collected from Laboratory of Bacteriology, NPUST. The bacterial strain was cultured on Wakimoto solid medium at 30 °C for three days. Wakimoto medium is a specific medium for Xoo bacteria, consist of 0.5 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 5 g of peptone, 20 g of sucrose, 15 g of agar in 1 L H_2O .

After three days of incubation, the bacteria was rinsed by sterilized distilled water and adjust the cell density to 10^8 cfu mL^{-1} . Then, 1 mL of bacterial suspension were cultured into 100 mL of Wakimoto liquid medium in 250 mL flask for 72 hours. Xoo bacterial suspension were filtered with 0.45 μm filter paper by sintered glass filter and with 0.2 μm filter paper sequentially. Then, the filtrate was filtered with 0.2 μm pore size again to obtain the cell filtrate for further experiments.

Xoo Screening

The cell suspension culture were separated into 24 microplate-well randomly. The research used 42 rice cell lines for screening. To screen the potential resistant cell lines, 10 % of Xoo filtrate were applied in each cell lines for 1 hours. The Reactive Oxygen Species (ROS) generation in each cell line was evaluated with 1 % of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA). The cells were imaged using a fluorescence microscope with a specific filter for green fluorescent protein (GFP). A higher fluorescence indicated a high ROS-producing in cells, while cell filtrate was recognized by rice cells.

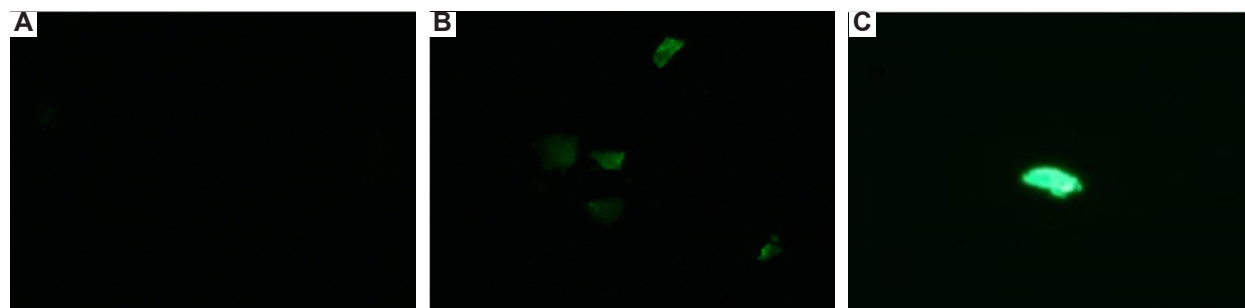
All cell lines were divided into three levels of cell brightness according to the fluorescence for determining their ROS producing levels (Fig. 1). Cell lines with higher ROS producing levels was regarded

as higher potential resistant of cell lines. Then, the cell lines were chosen for second screening. These cell lines were separated in several wells again, and the second run screening was performed with 10 % of Xoo filtrate for 1 hours under the same conditions. The highest ROS producing cell lines were chosen for the third screening, and the percentage of high ROS producing cell lines were calculated in each line

Table 1. Composition of media used in this study (mg L^{-1})

Compositions	CS-1 (Cheng & Shyu)*
NH_4NO_3	1000
KNO_3	1700
CaCl_2	500
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	330
KH_2PO_4	180
H_3BO_3	5
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	16
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	5.3
KI	0.8
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.3
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.3
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	30
Na2-EDTA	40
Inositol	100
Glycine	2
Glutamic acid	1
Aspartic	1
Thiamine-HCl	0.5
Pyridoxme-HCl	0.5
Nicotmic acid	0.5
Ascorbic acid	0.5
Folic acid	0.4
Biotin	0.1
Sucrose	30000

Remarks: * = CS-1 medium was developed by Cheng Chiu-Chsiung and Shyu Chen-Chuan, the Biotechnology Laboratory, Department of Plant Medicine, National Pingtung University of Science and Technology, Taiwan



Remarks: A. indicated none or weak ROS-producing cells; B. indicated medium ROS-producing cells; C. indicated strong ROS-producing cells

Fig. 1. Three distinct fluorescence level of cell to determine their resistant. Three distinct ROS-producing levels of rice cells. The ROS image (green) was performed by staining with H_2DCFDA as described in materials and methods

RESULTS AND DISCUSSION

Rice Cell Culture with Modification Medium

Plant Growth Regulator. In the first week, the cell number was not significantly different among treatments (Table 2). It was found that, the highest numbers of cell were produced in CS-1 medium with 4 mg L⁻¹ of 2,4-D. Further, the cells produced in the cultures were decreased when treated with 2 mg L⁻¹ and 6 mg L⁻¹ of 2,4-D. At the cellular level, 2,4-D as auxin control basic processes such as cell division and cell elongation. In this study, CS-1 medium with 4 mg L⁻¹ of 2,4-D showed a significant cell proliferation in 2 weeks of culture. However, 2.2 mg L⁻¹ 2,4-D in MS medium showed a faster growth for the initiation and growth of suspension cells (Htwe, Maziah, Ling, Zaman, & Zain, 2011).

Carbohydrate source. Carbohydrates played an important role in in vitro cultures as an energy and carbon source. Addition of glucose and fructose in CS-1 medium showed the higher proliferation than CS-1 supplemented with sucrose only (Table 3). CS-1 medium supplemented with 3 % sucrose + 0.5 % glucose + 0.05 % fructose shows the highest cell number for all week of culture. In the first week, the proliferation rate increased up to 56.67 % and in the third week increased up to 106.33 % (1 fold). It was similar to Ohira, Ikeda, & Ojima (1976), glucose and fructose supported cell growth as well as sucrose at the level of 2 % in B5 liquid medium. Sucrose, glucose and fructose have been used to support the growth, signaling molecules, differentiation, and metabolism of the cells in various tissue and cell cultures (Botha & O'Kennedy, 1998; Ong, Ling, Poosporagi, & Moosa, 2011).

Vitamin B1 (thiamine). The proliferation cell suspension culture in CS-1 medium supplemented with 3 % sucrose + 0.5 % glucose + 0.05 % fructose and treatment of 2.5 mg L⁻¹ thiamine was significantly increased almost 50 % in the first week culture (Table 4). However, the cell proliferation was not significantly different among all treatments in the second and third weeks of the culture. This result suggested that thiamine is necessary for rice cell suspension but has limited concentration. Removal of thiamine from the medium leads a rapid and complete decreasing of cell growth in soybean, tobacco and rice cells (Ohira, Ikeda, & Ojima, 1976; Ohira, Ojima, & Fujiwara, 1973).

Conditioned Medium (CM). In all of weeks of culture, the conditioned medium (1:1, 1:2, 1:3) showed a significant difference of proliferation rate of cell than fresh medium 1:0 (Fig. 2). The results suggested a beneficial effect of conditioned medium in cell proliferation. It might be caused by cell in primary culture was a critical step and easy to dead. Therefore the previous medium is still necessary for medium in new suspension culture stage for adaptation. This results supported the cell cultures secrete their metabolites to the suspension medium, where those secretion promote the initiation of new cell suspensions and growth regulators (Ben Amar *et al.*, 2007; van Hengel, Guzzo, van Kammen, & de Vries, 1998). In addition, the 1:1 of CM ratio treatment showed a higher initiation of cell proliferation in the first week of culture, 57.42 %. Therefore, the 1:1 conditioned medium ratio of CS-1 was recommended to rice suspension culture for further experiment.

Table 2. Effect of plant growth regulator (2,4-D) on proliferation rate of rice cell suspension in primary culture

PGR 2,4-D (mg L ⁻¹) ¹⁾	No. culture cells ($\times 10^5$ mL ⁻¹)	Cell number / week ($\times 10^5$ mL ⁻¹) Proliferation rate (%) ²⁾			
		1 ³⁾	2 ³⁾	3 ³⁾	4 ³⁾
2	1.41	1.59 a (12.65)	1.46 a (2.98)	1.12 a (-20.82)	1.20 a (-15.16)
4	1.41	1.50 a (6.05)	1.96 b (38.57)	1.22 a (-13.75)	1.58 b (12.17)
6	1.41	1.77 a (25.37)	1.34 a (-5.50)	1.19 a (-15.40)	1.31 ab (-7.15)

Remarks: ¹⁾ The suspension cells cultured in CS-1 conditioned medium (1:1) containing sucrose 3 % at darkness.

²⁾ Proliferation rate = (cell number each week - No. culture cells) / No. Culture cells $\times$ 100 %

³⁾ Data were analyzed by Duncan's Multiple Range Test, mean within a column followed by same letter are not significantly different at 5 % level of probability

Table 3. Effect of carbohydrate source on proliferation rate of rice cell suspension in primary culture

Medium ¹⁾	No. culture cells ($\times 10^5$ mL ⁻¹)	Cell number / week ($\times 10^5$ mL ⁻¹) Proliferation rate (%) ²⁾			
		1 ³⁾	2 ³⁾	3 ³⁾	4 ³⁾
CS-1	1.00	1.09 a (9,33)	1.24 a (24)	1.49 a (49)	1.22 a (22.33)
CS-1A	1.00	1.23 a (22,67)	1.43 a (43.33)	1.92 b (91.67)	1.22 a (22.33)
CS-1B	1.00	1.57 b (56,67)	1.71 b (71)	2.06 b (106.33)	1.54 (54)

Remarks: ¹⁾ The suspension cells cultured in CS-1/CS-1A/CS-1B conditioned medium (1:1) and 4 mg L⁻¹ 2,4-D at darkness

CS-1(control) : 3 % sucrose (as control)

CS-1A : 3 % sucrose, 0.3 % glucose, and 0.03 % fructose

CS-1B : 3 % sucrose, 0.5 % glucose, and 0.05 % fructo

²⁾ Proliferation rate = (cell number each week - No. culture cells) / No. culture cells $\times$ 100 %

³⁾ Data were analyzed by Duncan's Multiple Range Test, mean within a column followed by same letter are not significant different at 5 % level of probability

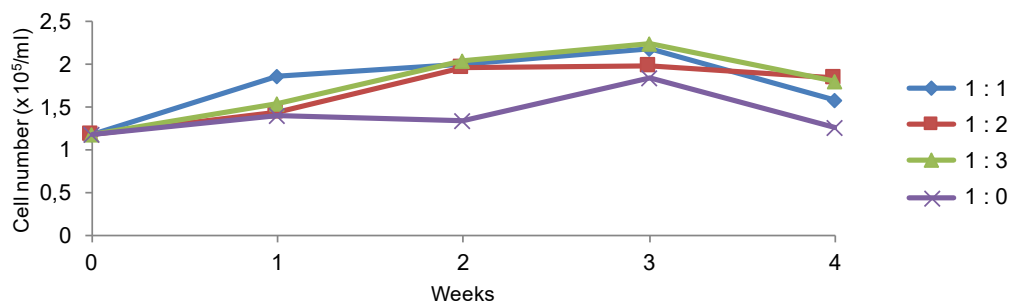
Table 4. Effect of vitamin B1 (thiamine) on proliferation rate of rice cell suspension in primary culture

Thiamine (mg L ⁻¹) ¹⁾	No. culture cells ($\times 10^5$ mL ⁻¹)	Cell number / week ($\times 10^5$ mL ⁻¹) Proliferation rate (%) ²⁾		
		1 ³⁾	2 ³⁾	3 ³⁾
0.5 (Control)	1.99	2.37 a (19.3)	2.87 a (44.30)	2.42 a (21.98)
2.5	1.99	2.93 b (47.48)	3.37 a (69.46)	2.77 a (39.43)
5	1.99	2.00 a (0.67)	2.86 a (44.13)	2.59 (30.20)

Remarks: ¹⁾ The suspension cells cultured in CS-1 conditioned medium (1:1) containing 3 % sucrose, 0.5 % glucose, 0.05 % fructose, and 4 mg L⁻¹ 2,4-D at darkness

²⁾ Proliferation rate = (Cell number each week - No. culture cells) / No. culture cells $\times$ 100 %

³⁾ Data were analyzed by Duncan's Multiple Range Test, mean within a column followed by same letter are not significantly different at 5 % level of probability

**Fig. 2.** Effect of Conditioned Medium (CM) on the proliferation rate of primary rice cell suspension culture

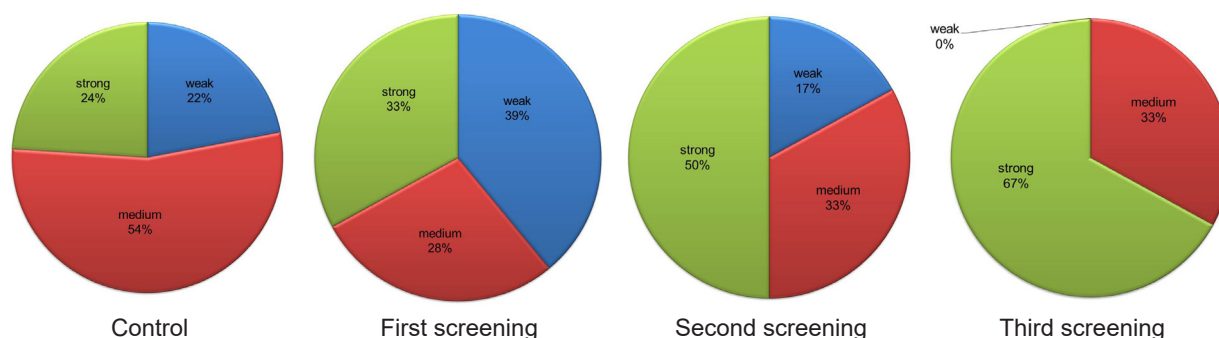


Fig. 3. ROS-producing ratios of rice cell lines during three times of screening. A, indicated the rice cells was not induce by 1 % of Xoo cell filtrate. B, indicated the ratios of ROS-producing rice cell lines in the first screening; C, indicated the ratios of ROS-producing rice cell lines in the second screening; D, indicated the ratios of ROS-producing rice cell lines in the third screening

Table 5. The population of ROS-producing cells in selected lines after three rounds of screening

No	Cell Lines	% Fluorescence level			ROS-producing level ¹⁾
		Strong	Medium	Weak	
	Control	9.00+12.45a ²⁾	62.33+ 9.25gh	28.67+ 7.941e	weak
1	D5-2-1	36.35+19.73 bcd	43.01+ 9.92abcde	20.63+14.06bcd	medium
2	D5-2-2	31.67+ 8.96bc	51.33+11.93defg	17.00+ 9.75cd	medium
3	D5-2-3	38.33+11.18bcde	58.33+11.76fgh	3.33+ 7.45ab	medium
4	D5-2-4	57.76+13.71fgh	39.74+13.07abcde	2.50+ 5.59a	strong
5	D5-2-5	46.00+19.21cdef	32.67+23.85abc	21.33+13.66de	strong
6	D5-2-6	35.67+18.24bcd	57.00+16.09efgh	7.33+10.11abc	medium
7	D5-3-1	66.27+10.87gh	26.15+ 4.34a	7.58+ 7.17abc	strong
8	D5-3-2	52.10+ 9.63defg	46.36+10.54cdefg	1.54+ 3.44a	strong
9	D5-3-3	45.00+13.23cdef	52.50+15.41defg	2.50+ 5.59a	medium
10	D5-3-4	52.55+15.82defg	45.22+15.16cdefg	2.22+ 4.97a	strong
11	D5-3-5	57.41+ 5.06fgh	40.72+ 5.40abcde	1.82+ 4.07a	strong
12	D5-3-6	59.76+10.92fgh	38.24+ 9.51abcd	2.00+ 4.47a	strong
13	D6-4-1	27.50+ 8.12b	58.33+11.76fgh	14.17+ 9.13bcd	medium
14	D6-4-2	51.44+ 1.97defg	45.23+ 5.03cdefg	3.33+ 4.71ab	strong
15	D6-4-3	51.35+ 5.08defg	44.62+ 7.42bcdef	4.03+ 4.73ab	strong
16	D6-4-4	45.24+13.89cdef	38.57+ 7.60abcd	16.19+ 16.7cd	strong
17	D6-4-5	50.00+ 7.07defg	45.00+ 9.84cdef	5.00+ 6.85ab	strong
18	D6-4-6	50.57+ 6.11defg	46.57+ 4.80cdefg	2.86+ 6.40ab	strong
19	D6-6-1	55.50+13.51efgh	43.07+13.16abcde	1.43+ 3.19a	strong
20	D6-6-2	53.24+12.79defg	43.90+11.41 bcdef	2.86+ 6.40ab	strong
21	D6-6-3	27.41+10.07b	70.77+10.83h	1.82+ 4.07a	medium
22	D6-6-4	72.50+11.31gh	27.45+11.31ab	0.00+ 0.00a	strong
23	D6-6-5	61.90+ 6.90fgh	38.10+ 6.90abcd	0.00+ 0.00a	strong
24	D6-6-6	47.50+13.05cdef	50.00+11.79cdefg	2.50+ 5.59a	medium

Remarks: ¹⁾ If the cells with strong and medium fluorescence were same or more than 75 % in population, the cell lines can be regarded as strong and medium of ROS-producing level. Afterwards, the ratio between strong and medium fluorescence were evaluated to determine the exactly ROS-producing level; ²⁾ Values in the table indicates means $\pm$ standard deviation. Data were analyzed by Duncan's Multiple Range Test, mean within a column followed by same letter are not significantly different at 5 % level of probability

Screening of Rice Resistant Cell Lines Against Xoo

In the first round of cell screening, 42 cell lines had been divided into three groups, including strong, medium, and weak of ROS-producing lines. The

result showed that 39 % of cell lines were having a weak ROS-producing, 28 % of cell lines had medium ROS-producing and 33 % of cell lines had a strong ROS-producing (Fig. 3). Then, two highest strong ROS-producing cell lines (D5 and D6) were cultured

and separated to 24-microplate again for the second-round screening. The result showed that around 50 % of cell lines were strong ROS-producing. Then, the four highest ROS-producing cell lines (D5-2, D5-3, D6-4, and D6-6) were separated and cultured again for the third-round screening. In the third-round screening, all of the cell lines were strong to medium of ROS-producing, while the cell lines with weak ROS-producing level was absent (Table 5). And totally, the ratio of strong ROS-producing cell lines in the third-rounds screening increased to 67%.

The screening strategy was based on ROS production in suspension cell after applied with Xoo filtrate. Since the cell filtrate containing the PAMPs from Xoo, the cell lines that more sensitive to PAMPs recognition would produce more ROS. Production of ROS in rice cell was performed by fluorescence observation with H₂DCFDA. This method has been shown in many studies to detect ROS generated in plant cells (Bi *et al.*, 2009; Eruslanov & Kusmartsev, 2010; Gomes, Fernandes, & Lima, 2005). The percentage of strong cell lines was higher in the pre-application with 1 % of Xoo cell filtrate than in control (Fig. 3). These results suggested that a high ROS-producing rice cell can produce higher amount of ROS while they recognized by Xoo. A rapid production of ROS is necessary in the mechanism of successful cellular responses of pathogen recognition to trigger plant defense response (Torres, Jones, & Dangl, 2006). Therefore, this research speculated that cell lines which can produce more ROS might have higher potential to become resistant plants.

Interestingly, the high ROS-producing cells can be selected by Xoo filtrate through multiple screenings. This strategy was conducted to the population of high ROS-producing cells which can be increased in 3 weeks. In addition, this research also observed that high ROS-producing featured in selected cell lines was stable during 3-time screening. Compare to the traditional plant breeding method, it a time-consuming for selection process (Ashraf & Akram, 2009), it usually takes 12-15 years to produce a new crop variety (Wieczorek & Wright, 2012). Therefore, this strategy may accelerate time period of screening resistant lines

CONCLUSION AND SUGGESTION

Based on the result that rice cell suspension in primary culture can be improved by CS-1 conditioned medium (fresh/spent medium ratio

1:1) containing 3 % sucrose, 0.5 % glucose, 0.05 % fructose, 2.5 mg L⁻¹ thiamine and 2 mg L⁻¹ 2,4-D. The rice cell lines with high ROS production may have potential of resistant cell lines against Xoo. Confirmation of disease resistance of novel cell lines to Xoo is worthwhile for more investigations.

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