Aging Effect on Accumulation of Sakuranetin in Paddy Plant after Elicitation by Silver Nitrate

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Abstract. Rice blast disease, caused by Pyricularia oryzae Cav. is the most serious fungal disease in rice crop, and this disease has caused significant yield losses worldwide. Sakuranetin which is a low molecular mass compound will be produced by paddy to protect paddy once the paddy is attacked by blast disease. A study was carry out on the effect of aging on production of phytoalexin (sakuranetin) after elicitation by silver nitrate. Paddy leaves were extracted after elicited by 1% silver nitrate solution for 12 weeks at two weeks interval using different leaves to determine the accumulation of sakuranetin. The highest accumulation of sakuranetin was in week six (1.32 µg ml-1) and the lowest in week 12 (0.37µg ml-1). The accumulation of sakuranetin increased from the four to six week and then decreased as the plant aged for the remaining six weeks. Moreover, week six had accumulation significantly different (p < 0.05) with the other four weeks. The regression test also showed that the production of sakuranetin was affected by aging.

Keywords: Sakuranetin; Rice blast; Yield increase.

INTRODUCTION

Rice or paddy (Oryza sativa) is one of the most important food in Asia. In Malaysia, rice is known as staple food. Up to date, rice blast disease, caused by Pyricularia oryzae Cav. (teleomorph: Magnaporthe grisea), is one of the most serious fungal disease in rice crop, and this disease has caused significant yield losses worldwide (Fabricio et al., 2003)

Phytoalexins are low molecular mass compounds absent in healthy plants and are produced only after plant is attacked by disease (Prinsloo and Meyer, 2006). Phytoalexins play important roles in suppressing the development of pathogens in host tissue. The synthesis and accumulation of rice plant phytoalexin can be induced not only through the invasion of the tissue by the rice blast fungus, but also through the exposure of plant tissue to abiotic agents such as sulfur dioxide (SO2) (Randeep et al., 2003), Jasmonic Acid (JA) (Prinsloo and Meyer, 2006) and UV-irradiation (Dillon et al., 1997). It has been shown that the production of phytoalexin in plant tissue is inversely proportional to increase in age.

Phytoalexins such as Oryzalexin E (Kato et al., 1993) D and S (Dillon et al., 1997), Sakuranetin (Nakazato et al., 2000), Phytocassane E (Koga et al., 1997), Momilactone A and B (Hisashi and Takeshi, 2005) can be found in paddy once paddy was infected by diseases. In rice plants, sakuranetin is a potent phytoalexin that was induced under a variety of abiotic and biotic stress stimuli (Ganesh et al., 2002).

MATERIALS AND METHODS

Plant Material and Growth Conditions. The seeds of JAYA variety paddy, soil and cotton were prepared. Soil and cotton were sterilized in autoclave. Then, the seeds were germinated on cotton and soaked in a container of water and incubated at 22°C – 25°C. Then the seedlings were transferred to a tray filled with soil and grown under natural sunlight.

Elicitation of the Paddy Leaf by Silver Nitrate. After 4, 6, 8, 10 and 12 weeks of paddy growth, 15g of paddy leaves were cut and soaked in 150 ml 1% of silver nitrate for six minute. The paddy leaves were incubated at 26°C for three and half days (84 hours).

Extraction of Sakuranetin from Rice Leaves. Fifteen gram of leaves were cut into small pieces (1 – 2 cm). The leaves were immersed in 150 ml of 70% aqueous methanol (at boiling point) in a conical flask and boiled for 10 minutes. After cooling, the flasks were left at room temperature, in the dark, for 24 hours. Then, the extracts were sieved through a filter paper and washed with 15 ml of 70% aqueous methanol. The extract was concentrated in Vacuum at 40°C.

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**Detection of Sakuranetin by Using Thin Layer Chromatography (TLC).** Fifteen milliliters of ethanol was added to the leaf extracts. The extracts were applied onto a Thin Layer Chromatography (TLC) plate (Merck kiesel 60 F254). The plates were developed in chromatography tanks pre-equilibrated with chloroform and ethanol (97:3) as the developing solvent. Then, the chromatograms were viewed under UV light (360 nm) to observe the presence of sakuranetin. Sakuranetin appeared in greenish fluorescence at Rf 0.09 (Atkinson and Blakeman 1982).

**Quantification of Sakuranetin by Spectrophotometry.** Sakuranetin which appeared under the UV light were marked by pencil and scrapped off from the TLC plate and dissolved into one ml of 100% aqueous methanol. The mixture was centrifuged for 20 minutes to extract sakuranetin from silica gel. Then, recovered sakuranetin was subjected to spectrophotometry with a maximum Absorbance at 357 nm (λmax) (Dillon et al. 1997). The concentrations of sakuranetin in units of Optical Density (OD) were converted to Molar concentration units using Lambert Beer Law (A = εℓc).

**RESULTS**

Figure 1 shows the concentration of sakuranetin accumulated in paddy for the past 12 weeks after elicitation with silver nitrate. The highest accumulation of sakuranetin was in week six (1.32 µg ml⁻¹) and the lowest in week 12 (0.37 µg ml⁻¹). The accumulation of sakuranetin increased from week four to six and then decreased as the plant aged for the remaining six weeks. However, there were no significant differences in sakuranetin accumulated at all ages except for week six, where plant showed an average of significantly higher sakuranetin concentration (50.15%) in comparison to other ages.

Regression analysis also showed that a quadratic polynomial fits best the relationship between the age of paddy plant and sakuranetin accumulation after silver nitrate elicitation. 58% of the variation in sakuranetin concentration was explained by the quadratic relationship (Data not shown).

Sakuranetin plays an important role in the mechanism of resistance of the rice plant against rice blast disease (Osamu et. al., 1992). Week six may be the most difficult time for Pyricularia oryzae Cav to invade paddy if sakuranetin involved in resistance against blast (Highest accumulation of sakuranetin).

There was an increase of sakuranetin in paddy leaves up to week six and decrease thereafter. Similar results were reported by Paxton and Chamberlain (1968) in soybean, where phytoalexin increased in the early stage of plant growth and decrease as the plant become older. Paxton and Chamberlain (1968) also reported that plant resistances decreased as soybean get older.

**DISCUSSION**

Paddy leaves appeared brown after elicitation with silver nitrate, indicating that necrosis or chlorosis of tissue and hypersensitive reaction (HR) may occur. HR is the result of quick mobilization of a cascade of defense responses by the affected and surrounding cells and the subsequent release of toxic compounds (phytoalexin) that often kill both the invaded and surrounding cells and, also, the pathogens (Agrios, 2005). The elicitation of older paddy leaves resulted in less browning compared to those younger leaves, showing that production of sakuranetin may decreased as the plant aged.

The result of this study showed that the production of sakuranetin in paddy decreased after week six. However, Choon et. al., (1999) revealed that the production of Momilactone A and B increased 50 days (seven weeks) after planting. The decrease in sakuranetin concentration in this study as opposed to the increase in Momilactone A and B may due to the resistance of other defense mechanisms such as morphological barriers and production of other antimicrobial compounds that took over the role of sakuranetin in defending paddy against whatever invasion.

**CONCLUSION**

The production of sakuranetin by paddy was influenced by plant age. Production of sakuranetin in week six was significantly higher (50.15 %) in average compared to other weeks.

**REFERENCES**

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