Influence of single nutrient element on 2-Acetyl-1-pyrroline contents in Thai fragrant rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 grown under soilless conditions

Sakon Monggoot[1], Phumon Sookwong [1,]*, Sugunya Mahatheeranont[1], Sawit Meechoui [2]

[1]*Rice Chemistry Research Laboratory, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[2]*Agriculture Technology and Research Institute, Rajamangala University of Technology Lanna, Lampang 52000, Thailand*

**e-mail: phumon.s@cmu.ac.th*

Abstract

Fragrant rice or aromatic rice is one of the most economically important products of Thailand. Fragrant rice contains 2-acetyl-1-pyrroline (2AP) as the major fragrant compound, which is genetically controlled. Its content is also affected by the environment, such as climate, production sites and soil conditions. Therefore, good management on cultivation conditions may enhance 2AP production in rice plants. Accordingly, the effect of some nutrient elements such as nitrogen (N), phosphorous (P), calcium (Ca), zinc (Zn), manganese (Mn) and magnesium (Mg) on 2AP production was studied. KDML 105 rice was grown under soilless conditions to prevent the effect of organic matters and possible redox reactions in soil. The N, P, Ca, Zn, Mn and Mg were added to 2, 4 and 8-fold from the Hoagland's standard nutrient solution. The content of 2AP in rice seeds was determined by using headspace sampling technique via gas chromatography–nitrogen phosphorus detector (HS-GC-NPD). 2AP concentration in the control was 1.42 ppm and it was increased up to 12.69, 16.68, 13.82, 13.06 ppm for 2-fold of N, P, Ca and Mn, respectively; 10.23 ppm for 4-fold of Zn and 12.83 ppm for 8-fold of Mg added from those contained in the standard nutrients solution. This finding indicates that adding suitable single nutrient element had enhancing effect on 2AP production in rice plants. Although, this study showed some positive effects that 2AP concentration was increased from control group by addition of nutrient elements but some additional studies on soil elements would be required to get better understanding on the subject.

Keywords: 2-Acetyl-1-pyrroline, Nutrient Elements, Soilless Conditions, KDML 105

Introduction

Rice (genus *Oryza*) is one of the most important plants that is manufactured to be basic food for world's population. Thailand, a massive rice exporter, is agriculturally economic country that uses the rice as an essential export product. Thai fragrant rice has a high value in many parts of Asia. Moreover, it has been widely accepted in Australia, USA, Australia and the Middle East. (Sakthivel et al. 2007) Fragrant rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 (KDML 105) is mostly cultivated in the Northern region and Tungkularonghai area as known as the best production site of high quality fragrant rice. 2-Acetyl-1-pyrroline (2AP) was firstly identified by Buttery and co-workers (Buttery et al. 1982) and suggested as the characteristic compound of aromatic rice. The 2AP production is genetically controlled but the concentration of is also actually affected by other factors such as environment, climate, location of fragrant rice production and nutrient elements. (Yoshihashi et al. 2004) Different locations provide different soil qualities that having different composition of nutritional elements. These findings imply that good management on cultivation conditions could enhance 2AP production in fragrant rice. Hoagland and Arnon developed hydroponic solution for study the stress of nutrient or physiology of plants. Accordingly, there are many affects on the hydroponic production system such as temperature or pH. The crop yield and quality is mostly considered from the effect of nutrient in solution. This is the first time that soilless technology was used to study the effect of nutrient elements on 2AP production in rice plants.

Methodology

Sample Preparation

The fragrant rice (KDML105), the seedlings are started in soil at the Center for Agricultural Resource Systems Research, Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand and, after 25 to 30 days, are moved and transplanted to hydroponics system (the number of clump of rice was limited up to 10). The rice was cultivated under hydroponics condition until it was approached to maturity stage; the seeds were harvested and stored in -18 °C.

Nutrient Elements for Hydroponics System

The nutrient solution reported by Hoagland and Arnon (1989) was applied in the present study as a control. The composition of nutrient solution used for growing rice in hydroponic system was listed in **Table 1**. All chemicals were dissolved in water before use.

Table 1: Composition of standard nutrient solution for plant (Hoagland and Arnon, 1989)

Chemical	Formula	Concentration
1. Calcium nitrate (15-0-0)	$Ca(NO_3)_2 \cdot 4H_2O$	15.25 kg in 25 litres H_2O
2. Potassium sulphate (0-0-50)	K_2SO_4	0.825 kg in 25 litres H_2O
3. Magnesium sulphate (50 %)	$MgSO_4 \cdot 7H_2O$	7.70 kg in 25 litres H_2O
4. Monopotassium phosphate (0-52-34)	KH_2PO_4	1.70 kg in 25 litres H_2O
5. Ammonium nitrate (Lab gr. 99 %)	NH_4NO_3	180 kg in 25 litres H_2O
6. Fe-EDTA (Lab gr. 99 %)	Fe-EDTA	5.6 g in 4 litres H_2O
7.Micronutrients dissolved in 2.5 litres H_2O		
- Manganese sulphate	$MnSO_4 \cdot 4H_2O$	12.45 g
- Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	2.65 g
- Copper sulphate	$CuSO_4 \cdot 5H_2O$	2.20 g
- Boric acid	H_3BO_3	9.50 g
- Ammonium molybdate	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.175 g
8. Sulfuric acid	H_2SO_4	200 cc in 1 litre H_2O

Table 2 shows the source of nutrient elements (N, P, Ca, Zn, Mn and Mg). The sources of nutrient provided the nutrient elements that were needed for the treatment. The chemical was prepared and added into the hydroponic pots to make final concentrations of 2, 4 and 8-fold standard nutrient solution.

Table 2: The source of selected nutrient elements for rice treatment

Nutrient Element	Source of Nutrient
N	NH_4NO_3
P	$NaH_2PO_4{\cdot}2H_2O$
Ca	$CaCl_2{\cdot}2H_2O$
Zn	$ZnSO_4{\cdot}7H_2O$
Mn	$MnSO_4{\cdot}H_2O$
Mg	$MgSO_4{\cdot}7H_2O$

Determination of 2AP in fragrant rice

Rice seeds (1.000 g) were ground and placed into a headspace vial. 1.0 µl of 2,6-dimethylpyridine (2,6-DMP) was added for used as the internal standard. After that, Headspace vial was sealed and analyzed by HS-GC. The method used for determination of 2AP was previously reported by Sriseadka et al. (2006). Based on 2AP concentration, this method was indicated to be effective when it used to evaluation of aroma quality in fragrant rice. (Sriseadka et al. 2006) Static HS-GC analysis was carried out using an Agilent Technologies (Wilmington, DE) model 6890N gas chromatograph equipped with an Agilent Technologies model G1888 headspace autosampler, and a nitrogen-phosphorus detector (NPD). The temperature was set at 275 °C. The column temperature was initially at 50 °C, and it was increased to 125 °C at a rate of 5 °C/min. The carrier gas flow rate was 5 mL/min.

Results

Percent Yield of Rice Seeds

The yield of rice was calculated from the number of filled and unfilled grain. It was found that the rice treated with 2-fold phosphorus provides the highest percentage of filled grain (73.28%), and 2, 4 and 8-fold addition of magnesium shows high percentages of filled grain (69.75, 71.06 and 72.44%), compared with other groups (**Figure 1**). The result shows the significantly different from control. The percentage of filled grain of controls is 65.75%.

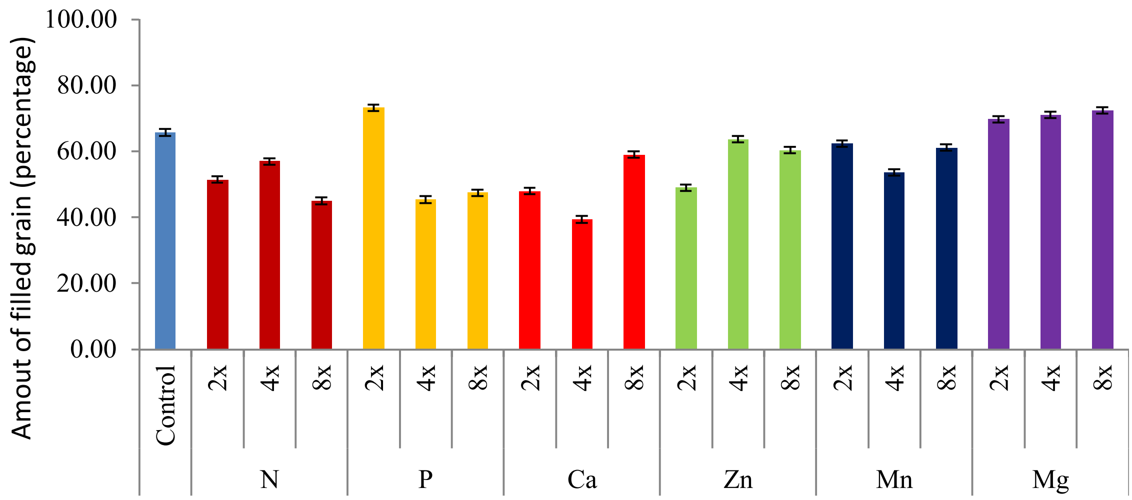

Figure 1: percentage of filled grain

The data of percentage yield shows the trend of information that scatter from each of treatment. The rice treated with magnesium show high percentage of filled grain followed by manganese, zinc, phosphorous, nitrogen and calcium, respectively. The result was considered by addition of 2, 4 and 8-fold of nutrient elements.

2AP Content in Rice Seed

From **Table 3**, 2AP contents from every treatment were greater than that of control (**Figure 2**). 2AP content in control was 1.42 ppm. 2AP in rice treated with 2, 4 and 8-fold of phosphorous (16.68, 7.70 and 13.12 ppm for 2, 4 and 8-fold, respectively) is higher than those of nitrogen (12.69, 1.41 and 11.99 ppm for 2, 4 and 8-fold, respectively). Considering, the group of minor nutrient treatment (Ca, Zn, Mn and Mg), the addition of Ca and Mn provided the greater content of 2AP when compare to the addition of Zn and Mn. 2AP content was up to 13.82, 10.07 and 12.83 ppm for 2, 4 and 8-fold of Ca added and 13.06, 11.99 and 11.43 ppm for 2, 4 and 8-fold of Mn added, respectively.

Table 3: The concentration of 2AP in rice seeds

Treatment	Concentration of 2AP in rice seeds, ppm
Control	1.42 ± 0.01
N (2x)	12.69 ± 0.19
N (4x)	1.41 ± 0.02
N (8x)	11.88 ± 0.32
P (2x)	16.68 ± 0.38
P (4x)	7.70 ± 0.16
P (8x)	13.12 ± 0.21
Ca (2x)	13.82 ± 0.32
Ca (4x)	10.70 ± 0.36
Ca (8x)	12.83 ± 0.38
Zn (2x)	7.34 ± 0.21
Zn (4x)	10.23 ± 0.14
Zn (8x)	8.46 ± 0.28
Mn (2x)	13.06 ± 0.33
Mn (4x)	11.99 ± 0.33
Mn (8x)	11.43 ± 0.29
Mg (2x)	1.83 ± 0.03
Mg (4x)	12.40 ± 0.40
Mg (8x)	12.83 ± 0.37

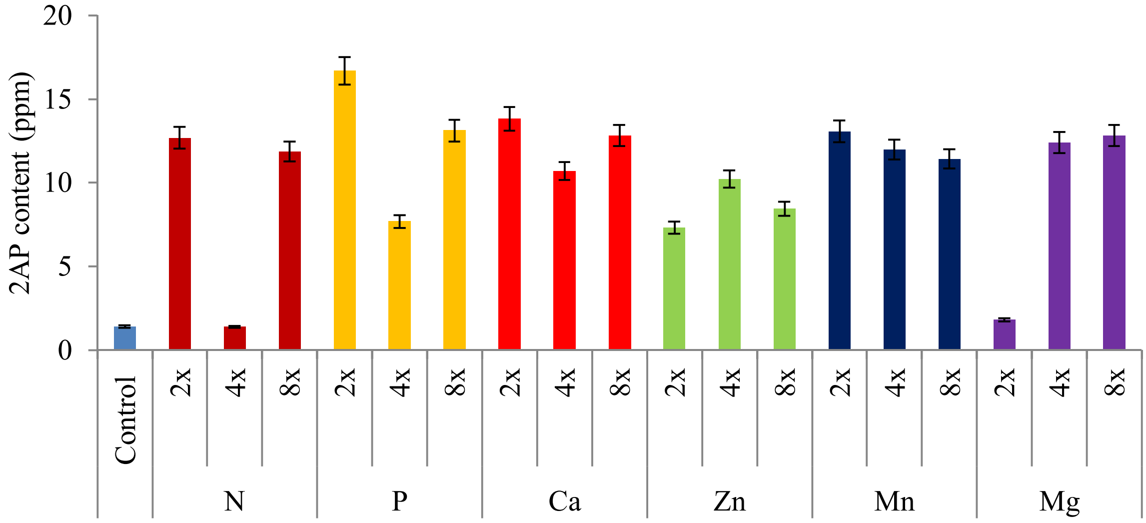

Figure 2: The concentration of 2AP in rice seeds

Discussion

The generally experiments mostly emphasize on effect of fertilizer on crop yield. Indira (2005) reported that nitrogen fertilizer could enhance the rice yield and Yoshihashi et al. (2004) reported that location for rice cultivation is affected on 2AP contents. Cultivation of rice in the hydroponic system could avoid the interferences from other factors such as and quality of soil in production site, climate change, water management, and chemical reaction (i.,e. redox reaction in soil). (Libia et al. 2012) In this present study, 6 single nutrient elements (N, P Ca, Zn, Mn and Mg) showed the positive effect on 2AP production that the concentration of 2 AP was increased up from those control. Various concentrations (2, 4 and 8-fold) of the single nutrient added were studied. It was found that rice plants could not produce their seeds when the concentrations of single nutrient solutions were below 2-fold and died when the concentrations above 8-fold from standard nutrient solution. The addition of phosphorous and calcium show the higher 2AP content than that of nitrogen for major nutrients. The rice that treated by manganese and magnesium presented the more content of 2AP than that of zinc for minor nutrients. In other hand, the highest percentage of filled grain was presented in magnesium treatment.

Conclusion

The experiment illustrates the effect of single element (N, P, Ca, Zn, Mn and Mg) addition on 2AP content in Thai fragrant rice. It was found that all with single element addition treatments show the positive effect. The addition of P (2-folds) showed the highest content of 2AP, while Mg addition (2, 4 and 8-fold) presents highest of percentage of filled grain. From the experiment it can be concluded that addition of single nutrient elements could enhance the yield production and 2AP contents in rice. The good management of nutrient elements in term of fertilizer formulation needed for 2AP production in rice would be required. In addition, beside the fertilizer, studies on agricultural management (such as water and soil technologies) would be conducted for the best production of the highest quality rice. A good management can also reduce the cost of fertilizer in fragrant rice production and conserve the quality of fragrant rice in term of flavor.

Acknowledgements

This project was supported by the Agricultural Research Development Agency (Public Organization) and Department of Chemistry, Faculty of Science, Chiang Mai University, Thailand.

References

Buttery R.G., Ling L.C., Juliano B.O. (1982) Cooked rice aroma and 2-acetyl-1-pyrroline in rice. Chem. Ind 24:958–959.

Fukai Y., Ishitani T. (2004) The reduction of old grain rice flavor by addition of aroma rice. J JPN Soc Food Sci 51:294–297.

Hoagland D.R. and Arnon D.I. (1950) The water-culture method for growing plants without soil. Circ. 347. Univ. of Calif. Agric. Exp. Station, Berkley.

Indira C. (2005) Effect of Nitrogen Fertilizer on Growth, Yield and Quality of Hybrid Rice (Oryza Sativa). J Cent Eur Agr 6(4):611–618.

Libia I., Trejo T., Fernando C., Gómez-Merino (2012). Nutrient Solutions for Hydroponic Systems, Hydroponics - A Standard Methodology for Plant Biological Researches, Dr. Toshiki Asao (Ed.), ISBN: 978–953–51–0386–8, InTech

Sakthivel K., Sundaram R.M., Shobharani N., Balachandran S.M., Neeraja C.N. (2009) Genetic and molecular basis of fragrance in rice. Biotech. Adv 27:468–473.

Sriseadka T., Wongpornchai S., Kitsawatpaiboon P. (2006) Rapid method for quantitative analysis of the aroma impact compound, 2-acetyl-1-pyrroline, in fragrant rice using automated headspace gas chromatography. J. Agric. Food. Chem 54:8183–8189.

Yoshihashi T., Nguyen T.T.H., Kabaki N. (2004) Area dependency of 2-acetyl-1-pyrroline content in an aromatic rice variety, Khao Dawk Mali 105. JARQ-JPN Agr Res Q 38:105–109.