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Prediction of 2-acetyl-1-pyrroline content in grains of Thai Jasmine rice based on planting condition, plant growth and yield component data using chemometrics☆

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ABSTRACT

The aim of this research was to simultaneously investigate the effects of nitrogen (N) fertilizer and salinity (NaCl) treatments on aromatic quality of *Oryza sativa* L. ssp. *indica* cv. Pathumthani 1 (PT1) rice grain. The levels of N and NaCl were designed based on a central composite design (CCD). During the cultivation, plant growth parameters such as number of tillers, plant height and root length were recorded. After the harvest, yield components including number of grains per panicle, panicle length, plant weight, shoot and root dry weights, number of panicle per plant, number of grains per plant and thousand grain weight were collected. The concentrations of 2-acetyl-1-pyrroline (2AP), a key odor-active compound, in the grains were analyzed using gas chromatography-nitrogen phosphorus detector (GC-NPD). Using partial least squares (PLS), the root mean square error of cross validation (RMSECV) value was 0.091 with the Q^2 value of 0.8470. Based on PLS coefficients and variable influence on projection (VIP) values, N, Na, the rice yield, the shoot dry weight and the number of tillers per plant were identified to have strong influence on the prediction of the 2AP content. The behaviors of the heavily influenced parameters were confirmed and visualized using self-organizing map (SOM).

1. Introduction

Rice is an essential food for people in many countries in particular in South and Southeast Asia. Regionally, Basmati from India and Jasmine from Thailand are among the well-known rice varieties not only because of their favorable textures but also the satisfactory aroma of the cooked rice [1]. Several studies have investigated on the chemistry of aroma and flavor in rice and found that 2-acetyl-1-pyrroline (2AP) was a key odor-active compound profoundly contributing to the pleasant scent of the cooked rice [2–6]. Therefore, the content of this aromatic component, 2AP, represents the aromatic characteristic of the rice grains and is considered as an important parameter for evaluating the quality of rice products.

Ideally, genetics has been regarded as a key factor in determining the rice aroma [7,8]. Besides the genetic factor, environmental factors, in particular salt stress and concentrations of some macronutrients, have been shown to affect the aromatic quality of rice [9]. Still, the mechanisms that explained these effects were not clearly defined. For example, Poonlaphdecha et al. [10] reported that salinity may have contributed to the concentrations of the aromatic flavors and some related amino acids in rice. However, the salt stress was reported to have no effect on the 2AP content in leaf tissue of Jasmine and Basmati cultivars [11]. In addition to the salt stress, nitrogen (N), a macronutrient, was possibly among the important factors in producing of the aroma in the aromatic rice. Yang et al. [12] reported that the higher the total N content in the soil, the better aromatic quality of the rice obtained. In contrast, Srivastava et al. [13] reported that aroma strength of a Basmati rice (Taraori variety) was inversely related to the nitrogen content in soil. However, this study did not focus on the concentration of 2AP and the aroma quality was monitored based on some other chemical compounds and a sensory test instead. The other study also reported that the rice samples did not differ in aroma or flavor although they were grown with different nitrogen rate applied [5].

Due to the inconsistent findings, there is no direct means to guarantee the rice yield with high aromatic quality. The variability in aromatic quality of the fragrant rice could be noticed although they were grown in traditional areas. At the present, it has not been easy to repeat the Jasmine rice crops with certain aromatic quality in the north and northeast of Thailand. This could be due to the fact that each of the potential factors effecting on the rice aroma was separately investigated. For example, the relationship between the nitrogen fertilizer and soil salinity that affects to the content of the main aromatic compound has not been yet investigated. Then, the effect of salt stress in relation to the concentration of nitrogen applied has not been confirmed.

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Chemometrics is a multivariate analysis that utilizes mathematical and statistical knowledge to extract the relevant information from chemical data. A variety of applications could employ chemometrics such as data exploratory, experimental design, pattern recognition/classification and multivariate calibration [14]. The aim of experimental design is to obtain the maximum information from designed dataset [15]. Experimental design can be useful for various purposes. For example, it is used for screening factors that are important to a chemical reaction. Based on the designed dataset, a quantitative model can be constructed to predict a response, leading to the possibility of defining the optimal condition. Since, the number of experiment is systematically designed, this allows enormous savings in experimental cost and time. A central composite design (CCD) is a popular experimental design used for examining the effects of several factors on a set of measured responses [16]. With a small number of systematically designed experiments, it is possible to obtain a mathematical framework describing how the values of the various factors affect to a studied response. Multivariate calibration methods such as partial least squares regression (PLS) could be used to relate the values of the studied factors or predictive data to the response. In addition to being used as a calibration model, the PLS regression coefficients could be used as a tool for revealing the significance of the variable and the interactions of the variables on the studied responses. However, PLS constructs a model based on an assumption that the data arises from a normal distribution. It basically expects a linear relationship among the dataset, direct or inverse relationships. Therefore, if the relationships of the studied factors could not be assumed, e.g. non-linear, their behaviors with respect to the response may not be correctly concluded. Self-organizing map (SOM) is an adaptive learning algorithm so it does not expect to fit the data into the expected distribution. This learning method could be used to reproduce the structure of the training samples and using visualization techniques such as U-matrix, supervised color shading and component plane, it is possible to investigate the behavior or the change in values of the predictive parameters with respect to the response [17,18].

In this research, chemometrics was used as a tool to provide a systematic analysis of data collected from a greenhouse experiment. A Jasmine rice variety, *Oryza sativa* L. ssp. *indica* cv. Pathumthani 1 (PT1), was cultivated in plastic pots using a modified Hoagland's nutrient solution with different rates of nitrogen fertilizer and salinity treatment designed based on the central composite design (CCD). Some plant growth and yield-related parameters were recorded and incorporated into a PLS model used for predicting 2AP content in the rice grains. Using PLS coefficients and variable influence on projection (VIP) values, important parameters effecting on the prediction of 2AP content were identified. The significance of the selected parameters and their behaviors effecting on the 2AP content in rice grains were confirmed and visualized using self-organizing map (SOM). This aspect will lead to insight into a possible management of salinity and fertilizer treatment during rice cultivation to obtain the grains with high aromatic quality.

2. Materials and methods

2.1. Statistical experimental design or design of experiment (DOE)

Design of experiment (DOE) systematically designs a set of experiments which maximizes the amount of information from the possible smallest number of experiments. In this work, DOE called central composite design (CCD) was used [19]. This design or sometimes called a response surface design comprises the advantages of a full factorial design, a star design and replicates at the centroid [16,19]. By adding the centroid and two other experimental points along each coordinate axis at the opposite sides of the origin with a distance equal to the semi-diagonal of the hyper cube of the factorial design, each factor of the CCD has five treatment levels. Using the experimental dataset obtained from the CCD, it is possible to generate a mathematical model that describes the relationship between the studied factors and the response properties. In addition, the established model could perform as a detailed quantitative model offering a predictive ability for the response based on the systematically designed data. In this work, the experiments were designed to vary the concentrations of nitrogen fertilizer and sodium salt. There were 9 treatments corresponding to 9 combinations of the studied factors. Each of the treatments had 5 replicate pots; therefore, the constructed design consisted of 45 experimental rice plants in total. Each of the rice plants was hydroponically grown in a plastic pot (10 L; 20 cm high). The rice cultivar was Pathumthani 1 (PT1), an improved fragrant temperate indica type (*Oryza sativa* L.) and non-photosensitive rice [20]. The nutrient solution used in this studied was modified based on Hoagland's nutrient standard solution [21]. Ammonium nitrate (NH_4NO_3) was used as the nitrogen source, while sodium chloride (NaCl) was used to induce saline condition in the experiments. The concentrations of nitrogen and sodium salt in nutrient solutions were shown in Table 1. Prior to the transplanting, the rice seeds were soaked in water for 24 h, and then incubated under moist and dark condition for 48 h. After that, each of the germinated seeds was transplanted in a sponge placed inside 1.5 in. thick plastic foam floating on the nutrient solution. During the first week of the transplanting, 50% concentration of the standard Hoagland's nutrient solution was used. Later, each of the experimental pots was treated with the nutrient solution according to the experimental design. The nutrient solutions were renewed weekly and the water level for each of the pots was maintained daily using fresh water if necessary.

The experiments were conducted in Mae Hia Agricultural Research, Demonstrative and Training Center, Chiang Mai University in Chiang Mai, Thailand, during the dry season (November 2013 to March 2014). The plant growth parameters including of number of tillers, plant height and root length were measured at various times (Table 2). After harvest, yield components such as number of grains per panicle, panicle length, plant weight, shoot and root dry weights, number of panicles per plant, number of grains per plant and thousand grains were collected (Table 3).

2.2. Determination of 2-acetyl-1-pyrroline (2AP) in rice grains

Prior to the analysis of 2AP content, the rice grains from each of the replicate samples were mixed together resulting in a total of 9 representative rice grain samples corresponding to the 9 experimental tests. Each rice sample was powdered using a Cyclotec TM 1093 mill with a 0.5 mm screen sieve (Foss, Höganäs, Sweden). One gram (1.00 g) of each sample was then put into a 20 mL headspace vial, this being followed by the addition of 1.00 μL of 0.50 mg/mL 2, 6-DMP in toluene. The headspace vial was then immediately sealed with a PTFE/silicone septum and aluminum crimp cap. It was shaken at a room temperature of 27 °C for 10 min prior to analysis by SHS-GC.

SHS-GC determination of 2AP was carried out using BRUKER technologies (Goes, Netherlands) model 450-GC gas chromatograph equipped with an QUMA headspace sampler model 40/111 (Wuppertal, Germany). A nitrogen-phosphorus detector (NPD) was used for

Table 1
Coded values and the concentrations of N and NaCl added to the standard Hoagland's nutrient solution for the central composite design (CCD).

Treatments	Coded values		Concentrations (ppm)	
	N	NaCl	N	NaCl
1	0.71	0.71	234	34
2	0.71	−0.71	234	6
3	−0.71	−0.71	150	6
4	−0.71	0.71	150	34
5	1	0	252	20
6	−1	0	132	20
7	0	1	192	40
8	0	−1	192	0
9	0	0	192	20