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Determination and Evaluation of Se-Rich High-Quality Rice Produced by Compound Nutrient Solution

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Abstract

Rice is one of the most important food crops in the world. Its biggest flaw is the low content of protein and essential amino acids, which severely limits its nutritional value. In order to produce high-quality rice with rich Se, we sprayed different concentrations of compound nutrient solution (containing Se (selenium), amino acid compound, zinc and boron) on the rice at different growth stages; and then determined the main nutrient content of their polished rice. The results showed that spraying low concentration compound nutrient solution (Each liter contained 20 mg of Se, 333 mg of complex amino acids, 33 mg of zinc and 33 mg of boron) to rice in the heading stage produced rice with the highest total starch and fat content and lower amylose content. Spraying high concentration compound nutrient solution to rice during the filling stage produced rice with the highest Se content. Multiple sprays of compound nutrient solution produced rice with low protein and low starch. Spraying low concentration compound nutrient solution on rice in milky stage significantly increased the content of protein, total starch, fat, all essential amino acids (Lysine increased by more than 57%), amylopectin and Se in rice; significantly reduced amylose content; significantly improved the nutritional value and taste quality of rice. The conclusion is that spraying low concentration compound nutrient solution on rice in the milky stage can produce rice with the highest content of protein and essential amino acids, higher content of total starch, fat and Se, and the lowest amylose content; significantly improve the nutritional value and taste quality of rice. The technical solution can comprehensively and effectively improve the nutritional value and flavor quality of rice, and has great development and application value.

Keywords: compound nutrient solution, high quality rice, high protein, high lysine, low amylose

1. Introduction

Rice is one of the most important food crops and staple food for more than half of the world's population. However, its biggest drawback is the low content of protein and essential amino acids, especially lysine, which severely limits its nutritional value. To improve the nutritional value of rice, researchers have made unremitting efforts (Yang et al., 2017; Tian et al., 2004; Xie et al., 2008). Li et al. (2008) analyzed the agrobacterium-mediated high lysine transgenic rice T4 generation. Their results showed that the lysine content of transgenic rice was only about 30% higher than that of the control rice variety. Hasanuzzamana et al. (2020) reported the recent advancement in the beneficial and harmful physiological role of Se (selenium) in plants under abiotic stresses. Recent studies show that Se treatment at a low concentration exerts positive effects on plant growth, development, and yield. Thus, Se may act as a vital element by altering several physiological and biochemical processes. Studies have shown that Se-rich foods have good health effects (Dinh et al., 2008; Santos et al., 2017). Fang et al. (2013) studied the effects of Se solution on the nutritional quality of rice grains. Chen

Xue et al. (2017) studied the effects of exogenous Se application on yield and quality of rice and Se distribution in plants. Cong (2011) studied the effects of Se-enriched fertilizer on rice yield and quality. Aboyeji et al. (2020) studied the effects of the zinc sulphate and boron-based foliar fertilizer on growth, yield, minerals, and heavy mtal composition of groundnut. Balawejder et al. (2019) studied the potential effects of foliar fertilizer based on calcined bones, boron and molybdenum on on maize grain production. Jiang et al. (2019) researched the effect of amino acid chelated Se nutrient liquid fertilizer on the yield and Se content of Baixiang 139 rice. Wang et al. (2003) reported the research progress of the biological effects of amino acids on higher plants. However, no major breakthrough has been made in the improvement of rice nutrients, especially essential amino acids such as lysine (Yang et al., 2017; Tian et al., 2004; Xie et al., 2008; Su et al., 2008; Shu et al., 2010). It is of great scientific significance and application value to improve the nutritional components of rice and increase the content of essential amino acids, especially lysine. To study the technology for producing high-quality rice with rich Se, we sprayed various concentrations of compound nutrient solution on Guanglu Dwarf-4 rice at different growth stages to study the effects of the fertilization period and concentrations of compound nutrient solution on the content of protein, starch, fat, amino acid and Se. We found that spraying low concentration compound nutrient solution on rice in the milky stage can significantly improve the nutritional value and taste quality of rice.

2. Methods

2.1 Test Materials and Main Reagents

The rice seed is Guanglu Dwarf-4. Each liter of compound nutrient solution contains 6 g Se, 100 g complex amino acids (Prepared with soybean meal), 10 g zinc, and 10 g boron. In application, the compound nutrient solution is diluted 300 times, 200 times and 100 times to prepare low concentration (C1, Each liter contained 20 mg of Se, 333 mg of complex amino acids, 33 mg of zinc and 33 mg of boron), medium concentration (C2, Each liter contained 30 mg of Se, 500 mg of complex amino acids, 50 mg of zinc and 50 mg of boron) and high concentration (C3, Each liter contained 60 mg of Se, 1000 mg of complex amino acids, 100 mg of zinc and 100 mg of boron) compound nutrient solutions, separately.

2.2 Field Experiment

Field experiments were performed by setting 3 parallel samples per group. The design scheme is shown in Appendix A. The area of each test field is 1 m × 4 m, and the line spacing is 200 mm.

The field experiment was conducted in Tuanhu Village, Shuangjiangkou Town, Ningxiang County, Hunan Province from June to September 2018. The test field is paddy soil. The climate was hot and humid during the experiment.

2.3 Spray Scheme for Compound Nutrient Solution

The growth period affecting the yield and quality of rice is mainly the heading stage, the filling stage and the milky stage. In the different growth stages of rice, 1-3 times of compound nutrient solution was sprayed on the leaf surface and ear of rice. The amount of spraying is determined by the formation of uniform mist droplets on the surface of rice leaves and ears. The specific spraying scheme is shown in Appendix B.

2.4 Rice Sample Preparation

When Guanglu Dwarf-4 rice is ripe, we harvested their seeds. Two kilograms of rice were obtained from each test group, and the rice was made into polished rice for nutrient composition determination according to Chinese GB/T 21719-2008.

2.5 Determination of Se Content in Rice

The pre-treatment of rice Se element is carried out according to Chinese GB 5009.93-2017 regulations. The Se content of rice was determined by PF7 hydride atomic fluorescence spectrophotometer (Beijing Puxi general Instrument Co., Ltd. in China) and SP-722E visible spectrophotometer (Shanghai spectrometer Co., Ltd. in China). Each sample was measured 3 times and averaged. The parameter settings for hydride generation-atomic fluorescence spectrometry are shown in Table 1.

Table 1. Parameter of determining Se elements by hydride generation-atomic fluorescence spectrometry

Photomultiplier tube negative high voltage	280 (V)	Atomizer height	8 mm
Lamp current	40 mA	Carrier gas	Argon
Delay time	4 s	Carrier gas flow	300 ml/min
Atomizer temperature	200 °C	Shielding gas flow	600 ml/min
Reading time	16 s		

2.6 Determination of Main Nutrients in Rice

Total starch was determined by Chinese GB 5009.9-2016 (acid hydrolysis). Amylose was determined by Chinese GB/T 15683-2008. Crude fat was determined by Chinese GB 5009.6—2016；Crude protein was determined by Chinese GB5009.5-2016. Each sample was measured 3 times and averaged.

2.7 Determination of Amino Acid Content of Rice Protein

Hunan Food Testing and Analysis Center was entrusted to determine the amino acids content of rice protein according to the requirements of GB 5009.124-2016 using Hitachi L-8900 amino acid automatic analyzer (Shanghai baihe instrument technology Co., Ltd. in China).

2.8 Data Processing

The data is processed with Microsoft Excel and SPSS 17.0 (International Business Machines Corporation (IBM), Armonk, New York, U.S.).

3. Results

The content of Se, amylose, total starch, protein and fat in rice samples was determined. The results are shown in Table 2.

Table 2. The main nutrient content of experimental rice

Sample	Se (μg/kg)	Amylose (%)	Total starch (%)	Protein (%)	Fat (%)
K0C0	16.75	18.10	72.36	8.54	0.83
K1C1	127.56	15.85	84.76	8.43	1.14
K1C2	149.73	17.95	81.67	8.17	0.99
K1C3	334.33	17.47	79.82	7.83	1.01
K2C1	647.92	16.96	77.01	8.66	0.88
K2C2	981.60	16.60	76.02	8.18	0.95
K2C3	1330.22	17.25	74.40	7.62	1.04
K3C1	450.56	15.79	74.48	10.05	1.10
K3C2	617.01	17.80	71.97	9.53	0.97
K3C3	1117.37	17.21	73.51	8.26	0.86
HFC1	575.86	17.22	73.69	7.94	1.10
HMC1	330.03	16.32	77.91	7.93	0.88
FMC1	757.44	17.47	72.27	7.94	0.67
HFMC	938.37	17.48	71.01	7.44	0.89

The amino acid contents of control rice and three high-protein rice with rich Se were determined. The results are shown in Table 3.

Table 3. Comparing the content of protein and amino acid in control rice and three high-protein rice with rich Se (g/100 g)§

Item	K0C0	K2C1	K2C1/K0C0	K3C1	K3C1/K0C0	K3C2	K3C2/K0C0
Protein	8.540±0.099	8.660±0.082	↑1%	10.050±0.132	↑18%**	9.530±0.104	↑12%**
Threonine	0.267± 0.015	0.297±0.016	↑11%	0.333±0.017	↑25%**	0.337±0.012	↑26%**
Valine	0.510±0.030	0.597±0.032	↑17%*	0.590±0.036	↑16%*	0.633±0.040	↑24%*
Methionine	0.170±0.011	0.153±0.010	↓10%	0.183±0.015	↑8%	0.187±0.012	↑10%
Isoleucine	0.283 ±0.015	0.330±0.017	↑17%*	0.460±0.027	↑63%**	0.477±0.029	↑69%**
Leucine	0.527±0.031	0.627±0.039	↑19%*	0.753 ±0.042	↑43%**	0.790±0.036	↑50%**
Phenylalanine	0.523 ±0.032	0.529±0.031	↑1%	0.627±0.035	↑20%*	0.610±0.020	↑9%*
Lysine	0.292 ±0.012	0.491±0.031	↑68%**	0.459±0.025	↑57%**	0.444±0.020	↑53%**
Tryptophan	0.353±0.015	0.450±0.020	↑28%**	0.473±0.025	↑34%**	0.483±0.023	↑37%**
Histidine	0.227±0.007	0.273 ±0.015	↑20%**	0.360 ±0.020	↑59%**	0.257±0.015	↑13%*
Cystine	0.042 ±0.020	0.054±0.031	↑27%**	0.058±0.030	↑38%**	0.067±0.035	↑59%**
Tyrosine	0.094±0.006	0.066±0.004	↓30%**	0.127±0.007	↑35%**	0.076±0.005	↑19%*
Aspartic acid	0.693±0.006	0.677±0.040	↓2%	0.633±0.039	↓9%	0.680±0.044	↓1%
Arginine	0.487±0.025	0.473±0.015	↓3%	0.660±0.036	↑36%**	0.623±0.038	↑28%**
Serine	0.547±0.031	0.440±0.027	↓20%*	0.430 ±0.026	↓21%**	0.497±0.025	↓9%
Glutamic acid	1.143±0.015	1.137±0.068	↓1%	1.273±0.076	↑11%	1.300±0.072	↑13%*
Glycine	0.407±0.021	0.400±0.020	↓2%	0.490±0.021	↑20%*	0.520±0.027	↑28%**
Alanine	0.637±0.038	0.603±0.035	↓5%	0.627±0.036	↓2%	0.813±0.044	↑28%**

Note. § ANOVA (Analysis of variance) was used to determine the significance of the differences in protein content of the samples.The T test was used to determine the significance of the difference between the amino acid content of the samples. * $P < 0.05$; ** $P < 0.01$.

4. Discussion

4.1 Effect of Concentration of Compound Nutrient Solution and Fertilization Periods on the Content of Se, Amylose and Total Starch in Rice

ANOVA (Analysis of variance) was used to analyze and compare the contents of Se, amylose and total starch in different rice in Table 2. The results are shown in Figure 1.

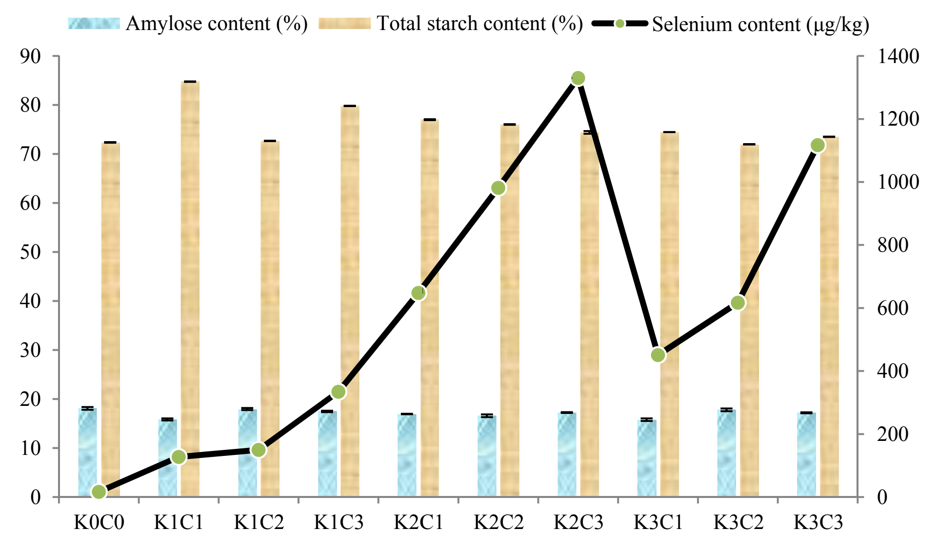

Figure 1. Effects of spraying different concentrations of compound nutrient solution on rice at different growth stages on Se, total starch and amylose content of rice

It can be seen from Table 2 and Figure 1 that the Se content of rice increased with the increase of concentration of compound nutrient solution. Se content of all rice was significantly higher than that of control rice ($P < 0.01$). K2C3 rice has the highest Se content, reaching 1330 μg/kg, which indicates that rice has the strongest ability to enrich Se during grain filling. K1C1 rice has the highest total starch content, up to 84.8%, and the lowest amylose content, only 15.8%, which indicates that spraying C1 compound nutrient solution at the heading stage can significantly increase the total starch content of rice and significantly reduce the amylose content ($P < 0.01$). However, when the concentration of compound nutrient solution increased, the total starch content decreased, the amylose increased. Yin et al. (2015) also discovered these phenomena. Because high concentrations of Se have toxic effects on plants (Hasanuzzamana et al., 2020). It can be seen that spraying C1 compound nutrient solution on rice at the heading stage can convert amylose to amylopectin, and increase the total starch content of rice. The content changes of these nutrients can significantly reduce the hardness of the cooked rice and improve its viscosity and taste quality.

4.2 Effect of Concentration of Compound Nutrient Solution and Fertilization Periods on the Content of Protein and Fat in Rice

ANOVA was used to analyze and compare the contents of Se, protein and fat in different rice in Table 2. The results are shown in Figure 2.

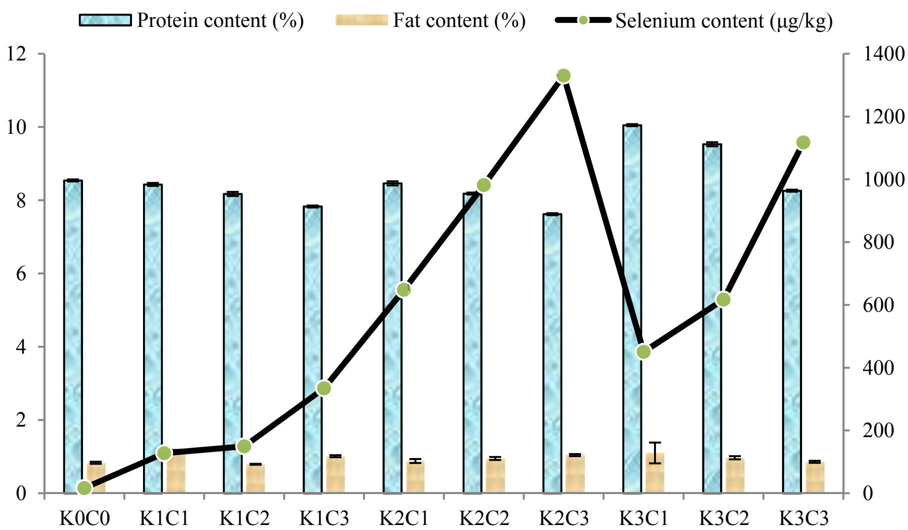

Figure 2. Effects of spraying different concentrations of compound nutrient solution on rice at different growth stages on Se, protein and fat content of rice

It can be seen from Table 2 and Figure 2 that praying C1 and C2 compound nutrient solution on rice at the milky stage can significantly increase the content of protein (up to 10.05% and 9.53%) and fat (up to 1.1% and 0.97%) in rice ($P < 0.05$). However, when the concentration of compound nutrient solution increases, the protein and fat content of rice decreases. This shows high concentration compound nutrient solution can significantly inhibit rice protein and fat synthesis. Spraying different concentrations of compound nutrient solution on rice during the heading stage and filling stage, the protein content of rice decreases or does not increase, but the fat content increased. Because an excessive Se concentration shows phytotoxicity associated with the overproduction of ROS as pro-oxidative Se activity, inhibition of the biosynthesis of photosynthetic pigments, and suppression of growth, developmental, and physiological processes (Hasanuzzamana et al., 2020).

4.3 Effects of Fertilization Times and Fertilization Periods on the Content of Rice Nutrients

ANOVA was used to analyze and compare the contents of Se, amylose, total starch, protein and fat in polished rice (Table 2) produced by spraying C1 compound nutrient solution 2-3 times on rice at 3 growth stages. The results are shown in Figure 3.

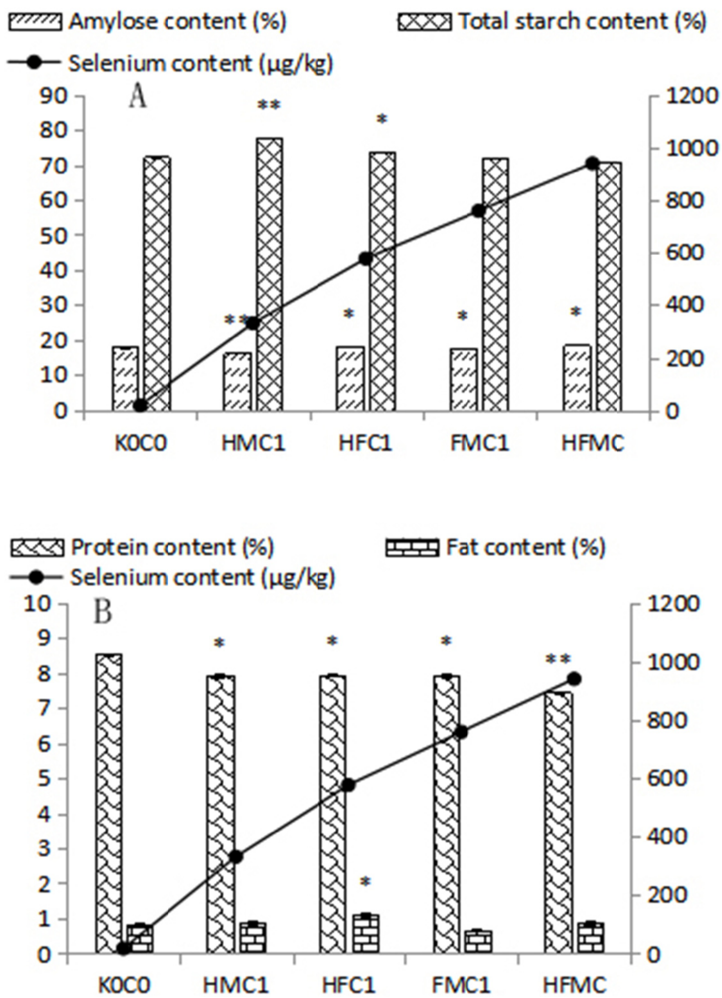

Figure 3. Effects of spraying 2-3 times of C1 compound nutrient solution on rice at 3 growth stages on the content of amylose, total starch, protein, fat and Se of rice

It can be seen from Table 2 and Figure 3A that spraying C1 compound nutrient solution on rice during heading and milk maturity can significantly increase the total starch content (77.9%) of rice (HMC1) ($P < 0.01$) and significantly reduce the amylose content (16.3%) ($P < 0.01$). In other combinations of fertilization, the amylose content was significantly reduced ($P < 0.05$), while the total starch content did not change significantly.

It can be seen from Table 2 and Figure 3B that spraying C1 compound nutrient solution 2-3 times on rice in 3 growth stages significantly reduces the protein content. The more the fertilization times, the more significant the protein content decreases. For instance, rice fertilized 3 times (HMND) had the lowest protein content (7.44%) ($P < 0.01$). Multiple applications of compound nutrient solution can inhibit protein synthesis in rice. Spraying C1 compound nutrient solution at the heading and filling stages of rice can significantly increase the fat content of rice. In other cases, the fat content does not increase. Kabata-Pendias (2010) reported Se bioconcentration ≥ 2 mg kg^{-1} dry weight in non-accumulator plants, like Arabidopsis thaliana, is toxic and can cause a 10 % decrease in the biomass without visible symptoms (Hasanuzzamana et al., 2020).

4.4 Effects of Concentration of Compound Nutrient Solution and Fertilization Periods on the Amino Acid Content of Rice Protein

It can be seen from Table 3 that the various amino acid content of the K3C1, K3C2 and K2C1 rice were significantly different from those of the control rice. The content of all essential amino acids and conditionally essential amino acids of K3C1 and K3C2 rice was significantly higher than that of control rice, and the lysine content was 53-68% higher ($p < 0.05$ or $p < 0.01$). Most essential amino acids of K2C1 rice are significantly higher than that of control rice, while its most non-essential amino acids are significantly lower than that of control rice. This suggests that spraying the C1 and C2 compound nutrient solution on rice in the milky stage and

C1 compound nutrient solution in the filling stage can significantly increase the content of essential amino acids and conditionally essential amino acids. The synthesis process of essential amino acids, especially lysine, is complicated and the synthesis speed is slow. Spraying the compound nutrient solution (such as C1) of suitable concentration in the milk maturity period after the filling period can increase the synthesis speed of essential amino acids and prolong their synthesis time, because Se, zinc and boron are components (active center) or activator of many metabolic enzymes (about 300 kinds), which can improve the activity of metabolic enzymes; and complex amino acids can provide nitrogen for the synthesis of essential amino acids (Hasanuzzamana et al., 2020; Fang et al., 2013; Balawejder et al., 2019; Niu et al., 2009; Aboyeji et al., 2020). The increase of essential amino acid content in rice, especially lysine content, can significantly improve the nutritional value of rice. Studies have shown that the increase of important nutrients in food can significantly promote the health of the body (Shen & Wu, 2017; Han et al., 2017; Wu et al., 2017). It can be seen that spraying a suitable concentration of the compound nutrient solution during a suitable growth period of rice is a good way to increase the essential amino acid content of rice and improve its nutritional value.

3.5 Mechanism of Compound Nutrient Solution Increasing the Content of Nutrients in Rice

This compound nutrient solution contains Se, zinc, boron and various amino acids. Se, zinc and boron are components or activators of many enzymes. They are related to the activities of many enzymes and have many biological functions (Fang et al., 2013; Balawejder et al., 2019; Niu et al., 2009; Aboyeji et al., 2020). Amino acids can be directly absorbed and utilized by rice, promote the metabolism of rice, and also have many biological functions (Jiang et al., 2019; Zhang et al., 2019; Wang et al., 2003). After spraying a low concentration (C1) of compound nutrient solution to rice ears and leaves during milk maturity, Se, zinc, boron and amino acids can be quickly absorbed by the rice. Through synergy, they can significantly increase the activity of many metabolic enzymes, promote photosynthesis in rice, increase the synthesis of essential amino acids, proteins, starch and fat, accelerate the conversion of amylose to amylopectin. In addition, fertilizing rice leaves and ears during the milky stage may extend the synthesis period of various nutrients in rice, especially essential amino acids (worth further study). Therefore, spraying this compound nutrient solution of appropriate concentration on rice during the milky stage can significantly increase the content of protein, starch, fat and essential amino acids, and significantly reduce amylose content, significantly improve the nutritional value and flavor of rice. Because a high concentration of Se has certain toxicity to animals and plants, spraying a high concentration of the compound nutrient solution or multiple sprays of the low concentration compound nutrient solution on rice can inhibit the synthesis of protein and starch and reduce their content (Hasanuzzamana et al., 2020).

In 2019, we used this technical solutions to plant 2,413 acres of rice in 21 villages in Ningxiang and Jingzhou counties in Hunan Province. The harvested selenium-rich rice is fragrant and delicious, and is generally welcomed by consumers, and its sales price has increased by more than 30%. The Se-rich high-quality rice produced by this technical scheme has high nutritional value and unique flavor. Its economic benefits have been significantly improved. This technical scheme surpasses the existing advanced technologies that produce rice, *e.g.*, high lysine transgenic technology, and has great promotion and application value (Yang et al., 2017; Xie et al., 2008; Fang et al., 2013; Jiang et al., 2019). We have achieved the great goals that agricultural scientists have not achieved for many years.

5. Conclusion

Spraying C1 compound nutrient solution on rice in the milky stage can produce rice (K3C1) with the highest content of protein, essential amino acids (especially lysine), higher content of total starch, fat and Se, and the lowest amylose content. The technical solution can comprehensively and effectively improve the nutritional value and flavor of rice, and has great development and application value.

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Abbreviations

GB = National standard; GB/T = Recommended national standard; K1 = Heading stage; K2 = Filling stage; K3 = Milky stage; C0 = Water solution; C1 = Low concentration of compound nutrient solution; C2 = Medium concentration of compound nutrient solution; C3 = High concentration of compound nutrient solution; K0C0 = Control; K1C1 = K1 + C1; K1C2 = K1 + C2; K1C3 = K1 + C3; K2C1 = K2 + C1; K2C2 = K2 + C2; the rest by analogy. HFC1 = K1 & K2 + C1; FMC1 = K2 & K3 + C1; HMC1 = K1 & K3 + C1; HFMC = K1 & K2 & K3 + C1; ANOVA = Analysis of variance.

Appendix A

Experimental Field Design

K1C1		K2C1		K3C1		K1C1		K2C1		K3C1
K1C2		K2C2		K3C2		K1C2		K2C2		K3C2
K1C3		K2C3		K3C3		K1C3		K2C3		K3C3
HFC1		HMC1		FMC1		HFMC		K0C0		HFC1
HMC1		FMC1		HFMC		K0C0		HFC1		HMC1
FMC1		HFMC		K0C0		K1C3		K2C3		K3C3
K1C1		K2C1		K3C1		K1C2		K2C2		K3C2

Appendix B

Spraying Scheme of Compound Nutrient Solution§

Group	Heading stage (K1)	Filling stage (K2)	Milky stage (K3)	Number of sprays
K0C0	C0	C0	C0	1
K1C1	C1	-	-	1
K1C2	C2	-	-	1
K1C3	C3	-	-	1
K2C1	-	C1	-	1
K2C2	-	C2	-	1
K2C3	-	C3	-	1
K3C1	-	-	C1	1
K3C2	-	-	C2	1
K3C3	-	-	C3	1
HFC1	C1	C1	-	2
HMC1	C1	-	C1	2
FMC1	-	C1	C1	2
HFMC	C1	C1	C1	3

Note. § K0C0 represent control group, C0, C1, C2, and C3 represent water solution, 20 mg/L, 30 mg/L and 60 mg/L compound nutrient solution, respectively. "-" means unsprayed compound nutrient solution. HFC1 = K1 & K2 + C1, HMC1 = K1 & K3 + C1, FMC1 = K2 & K3 + C1, HFMC = K1 & K2 & K3 + C1.