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Effects of Plant Growth Regulators on Starch Accumulation and Brewing Quality of Rice

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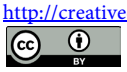

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Abstract

Rice is one of the main raw materials for rice wine and multi-grain Baijiu. The fine structure of rice starch and biochemical components such as protein, lipid and tannin content has significant effects on rice gelatinization and fermentation characteristics. However, the existing studies on plant growth regulators mainly focus on rice grain filling, rice quality and stress resistance, and there are few studies on the fine structure of rice starch, protein, lipid and tannin content, and its applicability to rice liquor-making. This paper reviews the effects of plant growth regulators on photosynthetic substance production, grain filling process and amylose and amylopectin formation in rice. At the same time, the effects of amylose, amylopectin content, proportion and structure, lipid and tannin content on rice gelatinization and the content of the aroma and flavor-producing substances in liquor were also reviewed. This can not only help the development of high-quality brewing functional rice industry and improve the utilization rate of rice, but also alleviate the shortage of high-quality brewing functional rice raw materials in China, improve the economic added value of rice, and provide new research directions for the research of plant growth regulators.

Subject Areas

Agricultural Science

Keywords

Review, Plant Growth Regulators, Amylose, Amylopectin, Fermentation, Pasting, Brewing Quality

1. Introduction

Rice is the most important food crop in China. Rice is the main grain for 2/3 of

the population in China, and the rice planting area accounts for about 27% of the domestic grain planting area [1]. The yield and quality of rice are directly related to the national economy and the people's livelihood. However, due to the lack of rural labor force and low economic benefits, farmers' enthusiasm for rice planting is generally insufficient, and rice production gradually evolves into "agriculture for women and the elderly", which seriously affects the sustainable development of rice industry [2] [3] [4]. Therefore, while ensuring food security, combined with local industrial needs, flexible transformation of rice commercial functions can effectively improve the added value of rice economy and improve farmers' planting enthusiasm.

Among many industries, baijiu industry, as an advantageous industry with unique Chinese characteristics, has a huge demand for rice and other brewing raw materials for a long time. Taking Yibin City, Sichuan Province, Luzhou City and Renhuai City, Guizhou Province in the "Golden Triangle" region of baijiu industry as examples, the annual demand for brewing raw materials can reach tens of millions of tons [5]. It can be seen that there is a good market for converting ordinary rice into brewing functional rice. However, local governments and baijiu enterprises have vigorously developed the construction of special grain production bases such as rice, the cultivation of rice for liquor has long depended on experience management. The lack of adaptability of rice starch digestibility, liquor yield and other brewing processing characteristics makes it difficult to meet the urgent needs of liquor-making enterprises for rice raw materials, which seriously restricts the high-quality development of liquor industry in China.

Plant growth regulators are a kind of agricultural inputs that regulate plant growth and development, which can affect a series of plant life processes from cell growth, division to rooting, germination, flowering, fruiting, maturation and abscission. At present, plant exogenous regulators have been widely used in various fields of agricultural production, and rice planting is also quite extensive, which has remarkable effects in promoting rice grain filling, yield formation and stress resistance regulation [6]. However, can plant growth regulators affect rice winemaking by regulating rice starch, protein, tannin and other biochemical components while regulating rice grain filling? At present, there is no relevant research. Based on this, this paper reviewed the effects of exogenous plant growth regulators on the photosynthetic substances and starch formation characteristics of rice at grain filling stage, as well as the effects of amylose, amylopectin, protein, lipid, tannin and other biochemical substances in rice on brew quality, so as to provide a basis for the development of grain-filling regulators of brew functional rice.

2. Plant Growth Regulators Regulate the Production of Photosynthetic Substances and Grain Filling Process in Rice

There have been many studies on regulating crop sugar and nitrogen metabol-

ism, photosynthetic function, dry matter transport and crop yield by exogenous chemical regulators. Spraying compound chemical regulators at heading stage of rice can significantly improve the appearance quality and milling quality of strong grains, and the expression of key enzymes in sucrose-starch metabolism in grains has been significantly improved [7]. Spraying certain concentration of plant growth regulators on rice leaves can improve the protective enzyme activity and rice yield [8]. Different plant growth regulators have different regulatory effects on nitrogen metabolism and photosynthetic characteristics of rice [9]. Tang *et al.* successfully developed an efficient rice quality improver with gibberellin (GA_3) as the main active component, which could slow down the decrease of chlorophyll content in flag leaves at the late growth stage of rice, thereby increasing rice yield, and improving the appearance quality and taste quality of grains [10].

The formation of rice starch properties depends on grain filling process. Rice yield is partly derived from the transport of dry matter stored in stems and leaves before flowering to grain, but more from photosynthetic matter production during grain filling [11] [12], Grain filling speed and filling days play a decisive role in yield [13]. Exogenous chemical regulators can further affect the formation of rice yield and quality by regulating the photosynthetic function of rice leaves and the transport of dry matter in plants [14]. Exogenous application of abscisic acid (ABA) in rice is conducive to the dry matter accumulation and grain filling of superior grains, while ethephon (ETH) can significantly promote the grain filling and dry matter accumulation of inferior grains [15]. Wang Linlin's study also had the same results. In addition, her also pointed out that after the treatment of plant growth regulators, the SPAD value and chlorophyll fluorescence parameters of rice increased at the late stage of grain filling, which was conducive to prolonging the photosynthetic time of leaves at the late stage of growth and promoting grain filling [16]. Therefore, the regulation of leaf photosynthetic function, dry matter accumulation and distribution, grain filling and yield formation of rice at grain filling stage can be achieved through exogenous regulators. However, there is no research on exogenous regulators specifically designed to regulate rice grain filling for alcoholic purposes.

3. Effects of Plant Growth Regulators on Amylose and Amylopectin Formation in Rice Grains

Amylose and amylopectin in rice were mainly formed during grain filling stage. The synthesis of amylopectin in rice grains is jointly regulated by a series of complex enzymatic reactions involved in carbon and nitrogen metabolism [17]. Studies have shown that ADPG pyrophosphorylase (AGPase), bound starch synthase (GBSS), soluble starch synthase (SSS), starch branching enzyme (SBE) and starch debranching enzyme (DBE) jointly regulate the formation of amylopectin. SBE I and SBE II are responsible for the synthesis of 70% and 30% of rice

endosperm amylopectin, respectively. SBE I mainly catalyze the formation of short chains, and SBE II catalyzes the formation of long chains [18]. In addition, regulators can promote starch and protein synthesis and change starch ratio. Wen *et al.* [19] showed that spraying ETH at jointing and flowering stages of wheat could significantly increase the contents of grain protein, total starch, amylose and amylopectin, and spraying GA_3 could also increase the grain protein content. Spraying 4.5 mg/L 6-benzylaminopurine (6-BA) on the 1st day before flowering or 1.0 mg/L PP333 on the 14th day after flowering can also significantly improve wheat yield and improve grain starch quality [20]. The mRNA expression of *OsGBSS1*, *OsSSSI*, *OsISA1*, *OsAGPL2* and *OsSBEIIb* were up-regulated and the mRNA expression of transcription factor *OsRSR1* was down-regulated by spraying exogenous 6-BA, ABA and increasing nitrogen fertilizer in rice. Spraying 6-BA and increasing nitrogen fertilizer significantly increased grain *OsGS1*; the gene expression of 3 was opposite when ABA was sprayed [22]. *OsGS1*; *3* Gene expression level is closely related to the activity of glutamine synthetase (GS) in grains [22], the increase of glutamine synthetase activity is conducive to rice grain filling [23]. At the same time, the expression of *OsGBSS1* gene in grain changed synchronously with amylose content [24]. This indicated that the increase of glutamine enzyme activity was conducive to the synthesis of amylose in grains, that is, it was conducive to the expression of *OsGBSS1* gene in grains. The expression levels of *OsSBEIIb* and *OsISA1* in grains affected the amylopectin content and fine structure. Fine structure and starch gelatinization characteristics could ultimately affect the quality of rice, and showed a positive correlation [25]. It can be seen that it is a feasible way to intervene the content and proportion of amylose and amylopectin in rice by exogenous plant growth regulators, and to change the starch characteristics and brewing applicability of rice.

4. The Quality of High-Quality Rice Is Beneficial to the Improvement of Brew Quality

With the improvement of people's consumption level, the demand for high quality rice is increasing. Rice quality is also regarded as a major limiting indicator in the certification of rice varieties to improve rice quality and market competitiveness, to improve rice quality and market competitiveness [26]. Rice is the main raw material for liquor-making, exploring the relationship between rice varieties, quality, processing and other factors and liquor brewing is of great significance to improve liquor quality, ensure the stability and quality safety of quality [27]. Rice cooking and eating quality significantly affect people's acceptance of rice [28], it is also an important factor affecting the yield of liquor ratio of rice. Among them, rice chalkiness has great influence on cooking and eating quality. The chalkiness is caused by light scattering due to insufficient grain filling of rice. The hardness of rice chalkiness is low and it is easy to break, which will lead to the decrease of head rice rate, more cracks in rice grains and the de-

crease of eating quality after cooking [29] [30] [31]. The main biochemical components in rice are starch, fat, protein and soluble sugar [32]. The contents of starch, protein, fat, tannin and minerals in rice grains are the key factors determining the cooking and eating quality of rice. At the same time, its content directly affects liquor quality and liquor yield. Li *et al.* [33] research finding that the base liquor quality of high-quality rice was better than that of ordinary rice, but the liquor yield was lower than that of ordinary rice. On this basis, Lin *et al.* [34] studies found that brown rice after crushing treatment is easy to cooking and pasting, and can improve the sensory flavor; the total acid and total ester content of high quality late indica rice base liquor were the highest, and the sensory quality of base liquor was excellent; the total acid and total ester content of high quality early indica rice were low, and sensory quality of base liquor was slightly poor; the contents of n-propanol, propionic acid and ethyl propyl ester in base liquor of common early indica rice were much higher than those of high quality early indica rice and high quality late indica rice, and the sensory quality of base liquor is better. In addition, starch (amylose, amylopectin), protein, lipid, tannin, crude fiber, ash and other biochemical substances are different, which will affect the quality of liquor [35].

5. Effects of Amylose and Amylopectin Content, Proportion and Structure on Rice Brewing Characteristics

Starch is the main material to produce alcohol, is the nutrition and energy of mold and yeast. In liquor brewing, starch must be gelatinized and hydrolyzed into monosaccharides to be utilized by microorganisms and to be fermented to produce flavor substances such as ethanol, lactic acid and esters [36]. It is generally believed that amylose is the key factor affecting rice quality, but there are still controversies. The base liquor brewed from rice is "liquor clean", free of impurities, and has little odor. The content of starch in grain and the ratio of amylose to amylopectin can directly affect the gelatinization characteristics of rice, the hydrolysis rate of enzymes in the process of brewing, the utilization rate of hydrolysis products by microorganisms and the quality of base liquor. The liquor yield and grade rate are significantly higher than those of conventional rice [37], and the fine structure traits of amylopectin such as branching morphology, proportion of long and short side chains played a key role in the brewing applicability of rice.

The content and proportion of amylose and amylopectin from different materials are different, which is one of the reasons for the difference of starch properties [38]. Rice amylose is generally divided into five grades according to its content: wax type 0% - 2%, very low type 5% - 12%, low type 12% - 20%, medium type 20% - 25%, high type 25% - 33%. Amylose mainly affects the eating quality of rice by interacting with lipids. The more long-chain content of amylose is, the easier it is to combine with lipids, which affects the water absorption of rice during cooking, and then affects its eating quality [39]. Reddy *et al.* [40] believed

that the determinant of rice quality was actually the fine structure of rice amylopectin. Through the comparison of sorghum brewing, it can be found that the amylopectin content in sorghum endosperm is high, and the viscosity is strong after cooking. It is easier to absorb water and expand, be gelatinized, and be decomposed and utilized by microorganisms [41] [42]. It is the preferred raw material for brewing high-quality Maotai-flavor Baijiu. At the same time, the higher the amylose content is, the closer the starch structure is, the longer the gelatinization time is, and the more fragrant the aroma is. At the same time, the higher the amylose, the tighter the starch structure, the longer the gelatinization time, and the more fragrant the aroma. The higher the amylopectin, the easier the gelatinization, the shorter the steaming time, the energy saving, and the higher the wine yield. In addition, too high gelatinization degree leads to excessive viscosity, reduces the oxygen mobility in the fermented grains, which is not conducive to the growth and reproduction of aerobic microorganisms, but reduces the brewing yield and has a light aroma [43] [44] [45]. It can be seen that the higher the content of amylopectin is not the more conducive to liquor-making.

In summary, the effects of amylose and amylopectin on liquor-making quality have a duality: the increase of amylose content will reduce the liquor yield on the one hand, and increase the aroma of liquor on the other hand; before the critical content, with the increase of the content, the yield of liquor was increased and the light aroma of liquor was given.

6. Protein Affected the Formation of Liquor Flavor Substances

In the process of brewing fermentation, the protein in the raw material is hydrolyzed by Aspergillus in the process of liquor production, which can provide nutrients for microbial reproduction such as yeast, and 30% - 50% (dry weight) of microbial cells is protein. When the protein content in the brewing raw materials is appropriate, the microorganisms grow vigorously and the enzyme activity is also high. The structural unit of protein is amino acid, which is the flavor substance of acid, sweet, bitter and fresh taste in Baijiu, and also the precursor of important flavor substances in Baijiu. The difference of amino acid content will directly affect the type and quantity of main flavor substances in the final base liquor [46]. Yeast cells can degrade amino acids to α-keto acids. α-keto acids are dehydrogenated and decarboxylated by enzymes to form aldehydes, which are further reduced to corresponding alcohols, and finally flavor esters are formed [47]. As shown in **Figure 1**, when amino acids were sufficient, yeast fermentation generated α-ketoacids by amino acid conversion through degradation metabolic pathway, followed by decarboxylation and dehydrogenation to generate higher alcohols with less one carbon atom; When amino acids are deficient, yeast cells synthesize essential amino acids through keto acid route through sugar-based synthetic metabolic pathway, and then synthesize their own cellular

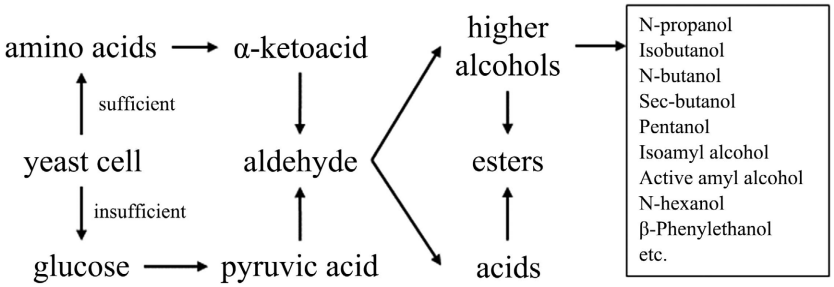

Figure 1. Effects of proteins and amino acids on higher alcohol metabolism and ester formation.

proteins. The intermediate *α*-keto acid is subjected to decarboxylation and dehydrogenation under the action of enzymes to produce corresponding higher alcohols [48] [49]. The mixture of higher alcohols produced by yeast metabolism is called fusel oil. When the amount of fusel oil is appropriate, the liquor will be round and full, and the taste will be soft and harmonious [50]. However, when the protein content is too high, the fusel oil generated by amino acids in the fermentation process is high, which will produce toxic effects and affect the quality of baijiu. In addition, amino acids could react with reducing sugars by Maillard reaction. After Amadori rearrangement and degradation under different pH conditions, pyrrole derivatives, pyrazine derivatives and furan derivatives were formed. Finally, further condensation and polymerization to form melanoidins and various aldehydes, ketones, acids and other components, giving liquor special flavor [51] [52]. At the same time, studies have also pointed out. That protein content is related to the viscosity of rice. The protein content has an inhibitory effect on the water absorption of rice grains, which makes the structure of rice grains more compact, and makes it difficult for water molecules to enter the pores between starch grains, resulting in long cooking time of rice or incomplete gelatinization of starch [53] [54]. It can be seen that protein affects rice gelatinization and the formation of liquor flavor substances, and controlling its content is of great significance to liquor production.

7. Effects of Lipids and Tannins on Rice Gelatinization and the Formation of Liquor Flavor Substances

Lipids in rice generally include starch lipids and non-starch lipids [53]. Rice lipids also have a non-negligible effect on the gelatinization characteristics of rice starch [54]. Lipids can form starch-lipid complex with starch, which changes the molecular and crystal structure of starch, thus affecting the solubility, gelatinization and viscosity of starch and other functional characteristics [55]. In addition, phospholipids and glycolipids in rice can act with starch, inhibit the water absorption and expansion of starch, and improve the gelatinization temperature of starch [56]. The gelatinization temperature of starch will affect the viscosity of rice. The higher the gelatinization temperature is, the worse the viscosity is. Moreover, the higher the gelatinization temperature is, the longer the gelatinization time is, that is, the more unfavorable it is to gelatinization, which is not

conducive to liquor production. On the other hand, lipids can produce a variety of volatile odors in the brew fermentation process, and then affect the flavor of liquor [57]. For example, the aliphatic ketones generated by the automatic oxidation of lipids are helpful for the formation of liquor flavor, but the low molecular aldehydes and ketones generated by the oxidation and decomposition of unsaturated fatty acids can cause rancidity, resulting in liquor body odor [58].

Tannin is a unique flavor and indispensable functional substance in Maotai-flavor liquor. Its content, structure and polymerization degree have direct or indirect effects on liquor quality [59]. Moderate tannin can inhibit the growth of miscellaneous bacteria; after fermentation, it can also be decomposed into soy sauce flavor precursors, such as syringic acid, ferulic acid and flavonoids, giving soy sauce flavor liquor unique flavor [60] [61]; excessive will make amylase, cellulase and other enzymes passivation, resulting in increased viscosity of distillers grains [62], affecting the metabolic activities of microorganisms affecting starch conversion, thus affecting the liquor yield and liquor quality. In addition, tannin can precipitate protein, so that it cannot be normal saccharification and fermentation, resulting in liquor astringency [58]. At the same time, too high tannin content will reduce the content of alcohol and ester flavor substances in the baijiu, and can inhibit the aroma stage of late fermentation, so that acid flavor substances cannot be transformed normally and ester flavor substances cannot be synthesized [63]. It can be seen from **Table 1** [64] that the effects of different concentrations of tannin on grain of cereal fermentation and brew quality were significantly different.

In summary, the effects of starch, protein, lipid and tannin components in rice grains on the liquor-making adaptability of rice are complex, but there are obvious correlations between each component, which can be seen in **Figure 2**.

Table 1. Effect of different tannin content on brew quality.

Tannin content (%)	Influence effect
<1%	The liquor yield and the high-quality product rate are low [65]
1.0% - 1.2%	The liquor yield and the high-quality product rate are high [65]
≥1.3%	The liquor yield decreases, but the high-quality product rate increases greatly [65]
1.4% - 1.7%	Suitable for brewing of Maotai-flavor baijiu [66]
0.5% - 1.5%	Suitable for brewing of light-flavor baijiu [35]
>1.4%	Affect yeast growth and inhibit liquor production [67]
<1.94%	The increase in tannin content increased the contents of ethyl acetate and ethyl caprylate, and vice versa decreased the contents of ethyl lactate and ethyl high-grade fatty acid [68].
>2%	Inhibition of most fungi growth [69]
>5%	Inhibition of all fungi growth [69]

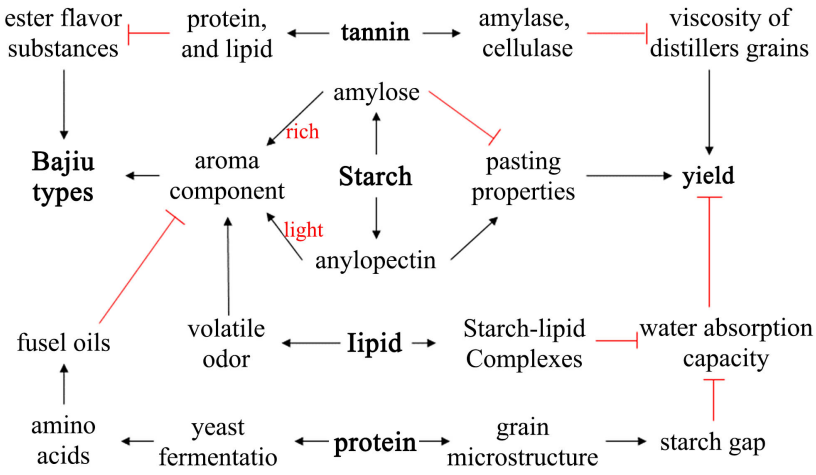

Figure 2. Effects of starch, protein, lipid and tannin components on brewing applicability of rice grains.

8. Prospect

How to further improve the brewing applicability of rice and improve the quality of baijiu from the source while ensuring the yield of raw materials is one of the feasible ways to promote the high-quality development of baijiu industry. Although many studies have confirmed that plant growth regulators can affect the grain filling process of strong and weak grains and the amylose and amylopectin contents in grains of rice, there are few studies on the effects of plant growth regulators on biochemical components such as proteins, lipids and tannins. The research on the adaptability of plant growth regulators to rice only focuses on increasing yield and improving resistance, but few studies have been conducted on the regulation of starch fine structure, protein, lipid and tannin content, and further the regulation of brewing adaptability of rice. Previous studies showed that proteins and lipids could indirectly affect rice brewing-making characteristics by affecting starch gelatinization characteristics. Future studies could therefore strengthen the following: 1) Analysis of the regulation of plant growth regulators on fine structure of amylose and the impact on the brewing applicability of rice; 2) To explore the response of grain starch synthase and leaf photosynthetic characteristic enzymes in different parts of rice during the filling stage, and then to analyze the regulatory effect of plant growth regulators on the rice filling stage; 3) Elucidating the characteristics of plant growth regulators regulating rice gelatinization, which in turn affected the brew quality of rice such as liquor yield, alcohol content, and content of the aroma and flavor-producing substances in baijiu; 4) Development of plant growth regulator products exclusively for brewing functional rice. The above research directions can not only improve the utilization rate of rice, but also alleviate the shortage of high-quality rice materials for brewing in China, and improve the economic added value of rice, providing a new research direction for the research of plant growth regulators.

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Conflicts of Interest

The authors declare no conflicts of interest.

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