DNA fingerprint and determination of functional components for rice with diabetes prevention

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Abstract. 31 Functional components of polished and brown rice and 50 SSR markers fingerprint for *boro* groups collected from 41 farmers from two village in Xinping county were investigated. The results as follows: We have bred first from rice landrace that Gongmi No. 3 with the highest resistant starch in the world, its resistant starch content of uncooked and cooked of polished rice are 8.0%~8.5% and 10%~13% respectively; meanwhile constructed the DNA fingerprinting of Xinpingzaoxian and Gongmi No.3 with high resistant starch based on 50 SSR markers. The cooked Xinpingzaoxian were steaming again and air-dried, then the resistant starch content were up to 12.2%; At present, Gongmi No.3 is the most ideal rice products to prevent chronic diseases in the World, especially for diabetes. The total amino acids, 4 amino acids and sulfur elements of brown and polished rice for Xinpingzaoxian with high resistant starch, which relatively distribute evenly in scytoblastema and endosperm, but 5 amino acids are unevenly distributed. Iron and zinc content of polished rice is 5.55% and 21.21% of brown rice, respectively.

Introduction

Diabetes mellitus (DM) is a major risk factor for cardiovascular disease, and the prevalence of DM is high and is increasing in China as well as in the world [1]. Diabetes of 366 million people worldwide caused an estimated US$465 billion in global healthcare expenditures in 2011, representing 11% of total worldwide healthcare expenditures in adults [2], and it has become one of the most challenging health problems of the 21st century. Rice is the staple food of more than 50 percent of the global population, functional rice is not only the largest functional foods in the diet, but also the new disease prevention strategies about the implementation of diet and effective way to solve the problem of global sub-health. The mean glycaemic index values of rice were 92 for glutinous rice, 86 for polished rice, 87 quality rice and 58 for Basmati rice, but 55 for brown rice and 35 high resistant starch rice as well as 19 for rice bran [3]. Resistant starch is starch that escapes from digestion in small intestine and ferments in large intestine, and it is useful for the prevention and treatment of diabetes, based on decrease blood glucose, cholesterol, and triglyceride, enhance the sensitivity of insulin, and reduce body weight [4].

Yunnan is not only one common sphere where the origin of human evolution is closely related to the origin of rice evolution, but also is the largest center of genetic diversity in the world [5]. A batch of rice landraces with high resistant starch were screened from 5285 accessions of Yunnan rice randrace, such Gongmi No.3 was screened from landrace Xinpingzaoxian in 1999, which suitable for planting in winter, accounts for about 9% of the Yunnan *boro* group. At home and abroad, the resistant starch content in rice is as high as 11%~13% has not been found. Therefore, it not only has important practical value to prevent chronic diseases such as diabetes and improve the quality of life, but also can breed function rice with high resistance starch to provide new materials, based on the determinate high resistant starch and mineral elements as well as amino acid content in rice from Yunnan rice landrace.

Materials and Methods

The equipment and determination of resistant starch(Gõni method,1999) [6] of 41 accessions in polished rice from *boro* of 41 farmers of two village in Xinping county, 9 mineral elements (P,K, Mg,S,Ca,Zn,Na,Mn,Fe,Cu,Ni,Mo and B by ICP-AES method) [7] and Vc as well as 17 amino acids (glycine, leucine, methionine, tyrosine, histidine, threonine, alanine acid, isoleucine, cystine, lysine, aspartic acid, valine, phenylalanine, proline, serine, glutamic acid and arginine by Hitachi-8800-automatic amino acid analyzer) content in mix samples of 41 accessions in rice see some literatures. Analysis of DNA fingerprint for of 50 SSR markers from 12 rice chromosomes of 41 accessions in rice see Zeng et al(2012) literature [8]. Select 50 pairs of SSR primers that distributed in 12 rice chromosomes and amplified effect better for bands Statistics, if have bands, mark with "1" , if not, mark with "0", use SPSS11.5 software on 50 SSR primer allele of Xinping early indica rice for cluster analysis.
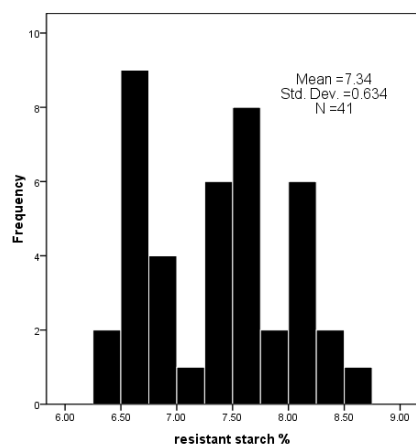
Results and discussion

Fig. 1 Resistant starch (%) in polished rice starch of 41 farmers for Xinpingzaoxian
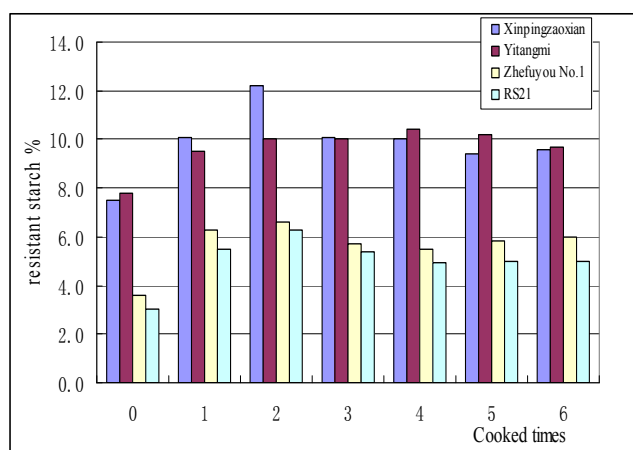

Fig. 2　The effect of cooking times and resistant content in 4 cultivars of functional rice

Table1. The difference of amino acid content between brown and polished rice of Xinpingzaoxian group

Amino acid	Brown %	Polished %	Amino acid	Brown %	Polished %	Amino acid	Brown %	Polished %
Tatals of amino acid	6.45	6.46	Glitamic acid	1.34	1.17	Lysine	0.29	0.30
Aspartic acid	0.64	0.63	Glycine	0.32	0.34	Threonine	0.27	0.24
Phenylalanine	0.35	0.38	Alanine	0.36	0.40	Serine	0.35	0.39
Isoleucine	0.27	0.26	Cystine	0.10	0.11	Histidine	0.13	0.17
Leucine	0.48	0.57	Valine	0.35	0.37	Arqinine	0.48	0.56
Tyrosine	0.21	0.29	Methionine	0.15	0.18	Proline	0.35	0.10

Resistant starch(7.34%±0.63%) in polish rice of *boro* group for randomly selected 41 farmers was domesticated from 1980 to 1999(see fig. 1), which show that this group (%) can be divided into three levels: high (6.3~7.0), higher (7.3~ 7.9) and the highest (8.0~8.5). New lines with stable green stem with resistant starch content among 8.0% ~ 8.5% in the highest level of polished rice were selected from Xinpingzaoxian group, which is named "Gongmi No. 3".　Resistant starch content in polished rice for Gongmi No. 3 after cooked 2 times is the highest, which show it belong to retrograded resistant starch with highest untilization value(See Fig. 2), and further disclose that there are greater difference resistant starch content between cooking times and genotypes among 4 cultivars with high resistant starch.

Table 1 shows that there are no obvious content difference in the total amount amino acids, 4 amino acids (aspartic acid, isoleucine, cystine and lysine) between brown and polished rice with Xinpingzaoxian group, which are distributed evenly in the scytoblastema and endosperm. 10 amino acids (especially leucine, tyrosine and arginine) between polished rice are higher than brown rice, only the glutamic acid, proline and threonine content of brown rice is obvious higher than polished rice. 5 amino acids (glutamic acid, proline, leucine, tyrosine and arginine) are distributed unevenly in scytoblastema and endosperm.

Table 2 shows that there are no obvious content difference for sulfur element between brown and polished rice of Xinpingzaoxian group, sulfur element is distributed evenly in the scytoblastema and endosperm. 5 mineral elements (P,K,Mg,Ca,Mn) of brown rice is about 2 times of polished rice, especially Fe and Zn content of polished rice is 5.55% and 21.21% of the brown rice.

Table 2. The mineral elements content differences of brown and polished rice

Element	brown $mg \cdot kg^{-1}$	polished $mg \cdot kg^{-1}$	polished /brown%	Element	brown $mg \cdot kg^{-1}$	polished $mg \cdot kg^{-1}$	polished /brown%
P	4000	2122	53.05	Mn	22.1	12.7	57.47
K	3000	1960	65.33	Fe	13.5	0.75	5.55
Mg	1300	780	60.00	Cu	12.7	6.01	47.3
S	950	993	104.53	Zn	86.3	18.3	21.21
Ca	154	84.8	55.06	Vc	10.0		

The figure 3 show that according to D = 2, based on 50 SSR markers cluster analysis of *boro* group of 41 farmers, this group can be divided into five groups: I: 50 SSR markers DNA fingerprint of Xinpingzaoxian for 27 farmers is same; II: 50 SSR markers DNA fingerprint of Xinpingzaoxian for 11 farmers is same; III: two farmers, the resistant starch content of ZZ is 8.2%; IV. Zhefuyou No.1 and V. RS21. Therefore, there are obvious difference among high resistant starch Xinping- zaoxian group, Gongmi No. 3, Zhefuyou 1 and RS21 that introduced from Zhejiang university. The DNA fingerprint between Xinpingzaoxian group and Gongmi 3 was constructed; In a word, the DNA fingerprint that was constructed by 50 SSR markers can effectively protect intellectual property of Gongmi No.3.
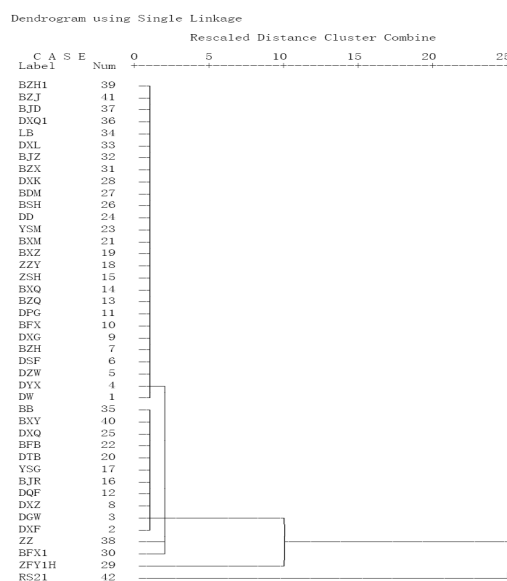

Fig.3 Cluster analysis of Xinpingzaoxian group and 3 cultivars with high resistant starch based 50 SSR markers

Conclusions

At home and abroad, this paper for the first time bred Gongmi No.3 with the highest resistant starch for natural polished rice as high as 8.0% ~ 8.5% (that is cooked rice resistant starch 10% ~ 13%) from Xinping *boro* groups. At present, resistant starch in polished rice of the RS111 as high as 7.54%, which get from Zhejiang university processing R7954 (resistant starch 1.80%) by space mutation and screen from 15000 single plant in M_2 poputation, however the AE as high as 8.25% , which is IRRI processing Kinmaze by chemical mutagens N - methyl - N - nitrosource and gain a AE mutant, then get from the hybridization of AE mutant and IR36[9]. In addition to construct DNA fingerprinting of Xinping boro group and Gongmi No.3 based on 50 SSR markers, it can provide valid evidence to effectively protect the intellectual property of Gongmi No.3.

The retrograded resistant starch for the best efficacy of dry rice of Gongmi No. 3 is 10%~13%, i.e. it is 55-fold than that of Diantun 502 with aromatic soft-rice (www.tiankan88.cn.alibaba.com). Gongmi No.3 as the raw material that produced in Ailao Mountain, Yungong brand "Shitangmi", there are 30 provinces in China, the trial of nearly more than 5000 chronic diseases patients, which show that Gongmi No.3 has a lots of diseases prevention, such as the control of postprandial blood glucose and diabetes prevention, anti-starvation, lowering blood lipid, weight control, and prevention of intestines diseases (constipation, enteritis, hemorrhoids),etc. which the total effective rate more than 95% (www.tiankan88.cn.alibaba.com). Therefore, Gongmi 3 has wide market prospect.

The total amount amino acids and 4 amino acids as well as Sulfur element of Xinping *boro* between polished and brown rice with high resistant starch are distributed evenly in the scytoblastema and endosperm, but 5 amino acids are distributed unevenly. This suggests that human beings from brown rice into polished rice may be lead to lack Fe and Zn for more than 2.5 billion people around the world.

Acknowledgments

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