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RESEARCH ARTICLE

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Starch granule protein (SGP) polymorphism in cultivated naked barley from Qinghai-Tibet Plateau in China and relationship between SGPs and starch/amylose content

Abstract Starch granule proteins (SGPs) are minor components bound with starch granule, whose variation could impact starch properties. This study investigated, for the first time, the variation of SGPs in the cultivated naked barley from Qinghai-Tibet Plateau in China. The relationship between SGPs and starch content was preliminarily dealt with. Ten major SGPs and 16 types of patterns were present in the 66 cultivated naked varieties, indicating that the SGPs in cultivated naked barley from Qinghai-Tibet Plateau in China are polymorphic. The SGPs of naked barley in Tibet and Sichuan were greatly different and the SGP patterns were specific to sampling regions. Significance test analysis demonstrated that the SGPs described in this study, except for SGP1, could be related with the variation of starch content in the different naked barleys.

Keywords cultivated naked barley, starch granule protein (SGP), polymorphism, starch content, Qinghai-Tibet Plateau in China

1 Introduction

Starch is a stable food in human and animal diets, and is also widely used as the raw material in industries. It is generally composed of amylose and amylopectin, existing as highly organized starch granule in various plants. The starch properties, including the total starch content, the ratio of amylose/amylopectin, the granule size distribution, and so on, vary greatly among plant species, cultivars or tissues. Such differences are responsible for the function and use of starch in each plant. The properties of starch are closely associated with a suite of biosynthesis involved in the synthesis of starch in plants (Mouille et al., 1996; Bernardo et al., 1997; Edwards et al., 1999; Yamamori et al., 2000). A number of mutants with modified starch biosynthesis and different starch properties were found in maize, wheat, potato and so on. It was also reported that a variety of barley starch mutants had significantly different starch properties from those of wild types. For example, an ADP-glucose pyrophosphorylase (AGPP) mutant in barley had a decrease of total starch content (Johnson et al., 2003); a barley mutant with a lower starch synthase SSI activity showed a reduction in the size of A-type starch granules and appeared in a unimodal granule size distribution (Schulman and Ahokas, 1990; Tyynela and Schulman, 1993; Tyynela et al., 1995); a SSII mutant had a 20% decrease of the amylopectin content, with shorter chain length and lower gelatinisation temperature (Morell et al., 2003); and a starch debranching enzyme (SDBE) mutant had no normal A- or B-type granules (Burton et al., 2002). Of course, high amylose mutants, as well as waxy barley, were also found, typically containing about 70% and 2%–10% of amylose (Morrison and Laignelet, 1983; Ishikawa et al., 1994). The availability of these mutants indicates that those enzymes taking part in starch biosynthesis greatly impact the starch properties in barley and the study of the variation of starch biosynthetic enzymes in different plant species or varieties could be beneficial to choosing the novel genotypes with favorable starch properties applicable for food and industrial uses.

The starch granule protein (SGP) is the minor component of the starch granule. It was previously reported that some SGPs were actively involved in the synthesis of starch (Denyer et al., 1995; Rahman et al., 1995). For example, the 60-kD SGP, a granule-bound starch synthase (GBSSI), was necessary for the synthesis of amylose (Shure et al., 1983); two bound starch granule proteins SGP-140 and SGP-145, preferentially associated with A-type starch granules, were

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different variants of SBEIc (Peng et al., 2000). There are few studies of SGP difference in barley, but the difference in polypeptide profiles for these proteins in wheat could be documented (Sulaiman and Morrison, 1990; Rahman et al., 1995; Yamamori and Endo, 1996). The objective of this study was to investigate the SGP variation in Cultivated Naked Barley from Qinghai-Tibet Plateau in China and the relationship between the SGPs and starch/amylose content, to choose novel starch mutants, to provide a theoretical basis for making good use of these rare naked barley resources, and to understand the enzymes mechanism in the process of starch biosynthesis.

2 Materials and methods

2.1 Plant materials

Sixty-six accessions of cultivated six-rowed naked barley (*Hordeum vulgare* L.) from Qinghai-Tibet Plateau in China were studied. Among the materials, 46 accessions were collected from Tibet, which were provided by Tibet Academy of Agricultural and Animal Husbandry Sciences, and 20 were from Sichuan, which were provided by the Agricultural Institute of Aba Autonomous Prefecture, Sichuan. Concerning growth habits, 56 accessions were of spring types and 10 of winter types. The altitudes of the sampling sites were from 1600 m to 3850 m above sea level (Table 1). The cultivar Chinese Spring was taken as control.

2.2 Methods

The SGPs in naked barley were separated with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique using the method as described by Pan et al. (2000). Bands were identified using the software Quantity one 4.5.0 and numbered consecutively. The total starch and amylose were determined according to the national standard method GB5006-85 and GB/T 15683, respectively. The index of genetic diversity was calculated using the POPGENE 1.31 software (Yeh et al., 1999). A dendrogram was produced using the program NTSYSpc 2.0 (Rohlf, 1998). Statistical analyses were performed using the software package SPSS 10.01. The least significant difference (LSD) at the 5% probability level was calculated, and a significance difference among experimental materials with different SGP patterns was observed.

3 Results

3.1 SGPs polymorphism in naked barley

Figure 1 shows that the SGPs in naked barley were clearly separated and a wide range of variation was

Table 1 List of 66 cultivated hulless cultivars from Qinghai-Tibet Plateau in China used in this study

sampling regions	accession name	number of accession	growth habit	altitude/m
Tibet	Zangqing 1	TZ01	Spring	3700
	Zangqing 2	TZ02	Spring	3700
	Zangqing2 1	TZ03	Spring	3700
	Zangqing 80	TZ04	Spring	3700
	Zangqing 85	TZ05	Spring	3700
	Zangqing 3179	TZ06	Spring	3700
	Zangqing 311	TZ08	Spring	3700
	Zangqing 148	TZ09	Spring	3700
	Zangqing 320	TZ10	Spring	3700
	815078	TZ11	Spring	3700
	940690	TZ12	Spring	3700
	QB01	TZ13	Spring	3850
	QB02	TZ14	Spring	3850
	QB09	TZ15	Spring	3850
	QB24	TZ16	Spring	3850
	QB16	TZ17	Spring	3850
	QB25	TZ18	Spring	3850
	QB28	TZ19	Spring	3850
	Ximala 2	TZ20	Spring	3600
	Ximala 4	TZ21	Spring	3600
	Ximala 6	TZ22	Spring	3600
	Ximala 8	TZ23	Spring	3600
	Ximala 9	TZ24	Spring	3600
	Ximala 10	TZ25	Spring	3600
	Ximala 11	TZ26	Spring	3600
	Ximala 15	TZ27	Spring	3600
	Ximala 16	TZ28	Spring	3600
	Ximala 19	TZ29	Spring	3600
	Ximala 42	TZ30	Spring	3600
	Shanqing 7	TZ31	Spring	3600
	Lashagoumang	TZ32	Spring	3700
	Lashaziqingke	TZ33	Spring	3700
	Dongqing 1	TZ34	Winter	3700
	Dongqing 8	TZ35	Winter	3700
	Dongqing 11	TZ36	Winter	3700
	Dongqing 15	TZ37	Winter	3700
	Dongqing 16	TZ38	Winter	3700
	96-971800	TZ39	Winter	3700
	94-95-955	TZ40		
	WB21	TZ43	Winter	3850
	WB19-97	TZ45	Winter	3850
	WB07-97	TZ46	Winter	3850
	Zang 434	TZ78	Spring	3700
	Zang 447	TZ79	Spring	3700
	Zangqing 25	TZ87	Spring	3700
	Pinbi14	TZ54	Winter	3700
	Beiqing 3	TZ55	Spring	3700
Sichuan	Handiziqingke	SZ48	Spring	3300
	Litangbaisanshe	SZ50	Spring	3600
	Chunqingke	SZ51	Spring	3300
	Changtichunqingke	SZ53	Spring	2300
	Abasiqingbailiuling	SZ56	Spring	3300
	Abashandan	SZ58	Spring	3300
	Hongyuan 86	SZ59	Spring	3400
	Zhanglabaiqingke	SZ60	Spring	3100
	Rangtanghuangqingke	SZ62	Spring	3000
	Xiaojinrilongziqingke	SZ64	Spring	2000
	Luobozailiuling	SZ65	Spring	1900
	Jinchuanliulingqingke	SZ66	Spring	2000
	Zumujiaoliulingqingke	SZ68		2300
	Baxihuiqingke	SZ69	Spring	3100

(*Continued*)

sampling regions	accession name	number of accession	growth habit	altitude/m
	Aqing 5	SZ71	Spring	3000
	97039-2	SZ74	Spring	3450
	Daanbaheiqingke	SZ75	Spring	2400
	Qianningqingke	SZ77	Spring	
	Kangqing 3	SZ81	Spring	3450
	97-9	SZ84	Spring	3450

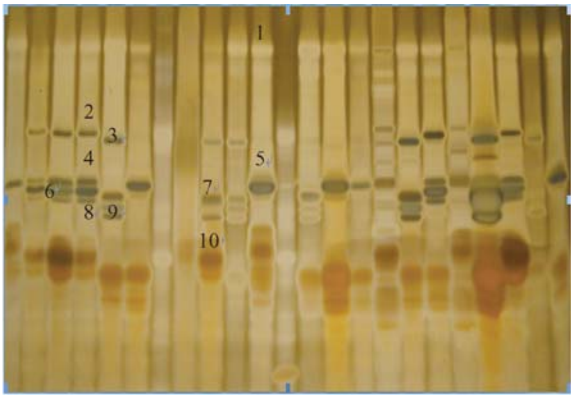

Fig. 1 SDS-PAGE for SGPs in naked barley

observed. In total, there were 10 major SGP bands identified, 9 bands of which were polymeric, except for SGP1. The frequency of the polymeric bands ranged from 16.67%–48.48%, and the bands SGP5 and SGP9 occurred the least (Table 2). Generally, the SGP electrophoretic analysis revealed 16 patterns of bands among the 66 tested accessions, and 9 of them were unique (Fig. 2, Table 3). Each of the remaining 7 patterns included 2–19 accessions, and Types 2, 3 and 8 were common, representing 10, 13 and 19 accessions, respectively. The number of bands per line ranged from 2 to 5, and those accessions with 4–5 bands were the most frequent, accounting for 33.33% and 43.94%, respectively (Table 4).

Table 2 Frequency of SGP bands identified within the different population studied

SGP	Tibet/%	Sichuan/%	total/%
SGP1	100.00	100.00	100.00
SGP2	34.78	55.00	40.91
SGP3	39.13	35.00	37.88
SGP4	28.26	30.00	28.79
SGP5	26.09	5.00	19.70
SGP6	30.43	60.00	39.39
SGP7	41.30	35.00	39.39
SGP8	43.49	60.00	48.48
SGP9	19.57	10.00	16.67
SGP10	41.30	35.00	39.39

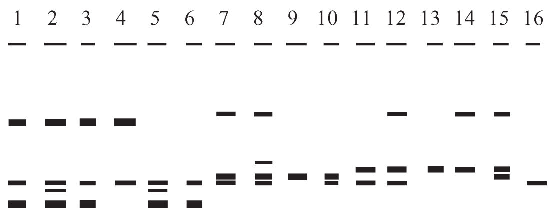

Fig. 2 Types of patterns for SGPs

3.2 Comparison of the SGP variation between Sichuan and Tibet accessions

Both Tibet and Sichuan accessions contained 10 major SGP bands, and there were no specific band associated with the collecting regions. Each of the SGP bands presented different frequency in these two populations.

Table 3 Types of patterns of SGPs in the naked barley

patterns	band composition	No. of accession	No. of accession	No. of bands
1	SGP 1+3+8+10	TZ45	1	4
2	SGP1+3+7+9+10	TZ46 SZ81 SZ51 TZ2 TZ8 TZ30 TZ1 TZ3 TZ29 TZ33	10	5
3	SGP1+3+7+10	TZ55 TZ43 SZ60 SZ69 SZ77 TZ79 SZ84 SZ68 TZ9 TZ26 TZ17 TZ19 TZ23	13	4
4	SGP1+3+7	TZ38	1	3
5	SGP1+7+9+10	TZ14	1	4
6	SGP1+7+10	TZ32	1	3
7	SGP1+2+6+8	SZ50 SZ58 SZ65 SZ59	4	4
8	SGP1+2+4+6+8	SZ64 TZ54 SZ74 SZ56 SZ75 TZ20 TZ31 SZ62 SZ66 TZ5 TZ6 TZ27 TZ28 TZ39 TZ13 TZ35 TZ36 TZ37 TZ40	19	5
9	SGP1+6	TZ78	1	2
10	SGP1+6+8	SZ71	1	3
11	SGP1+5+8	TZ4 TZ12 TZ22 TZ21	4	3
12	SGP1+2+5+8	TZ16 TZ25	2	4
13	SGP1+5	TZ24 TZ18 TZ15 TZ10 TZ34	5	2
14	SGP1+2+5	TZ11	1	3
15	SGP1+2+5+6	SZ48	1	4
16	SGP1+8	SZ53	1	2

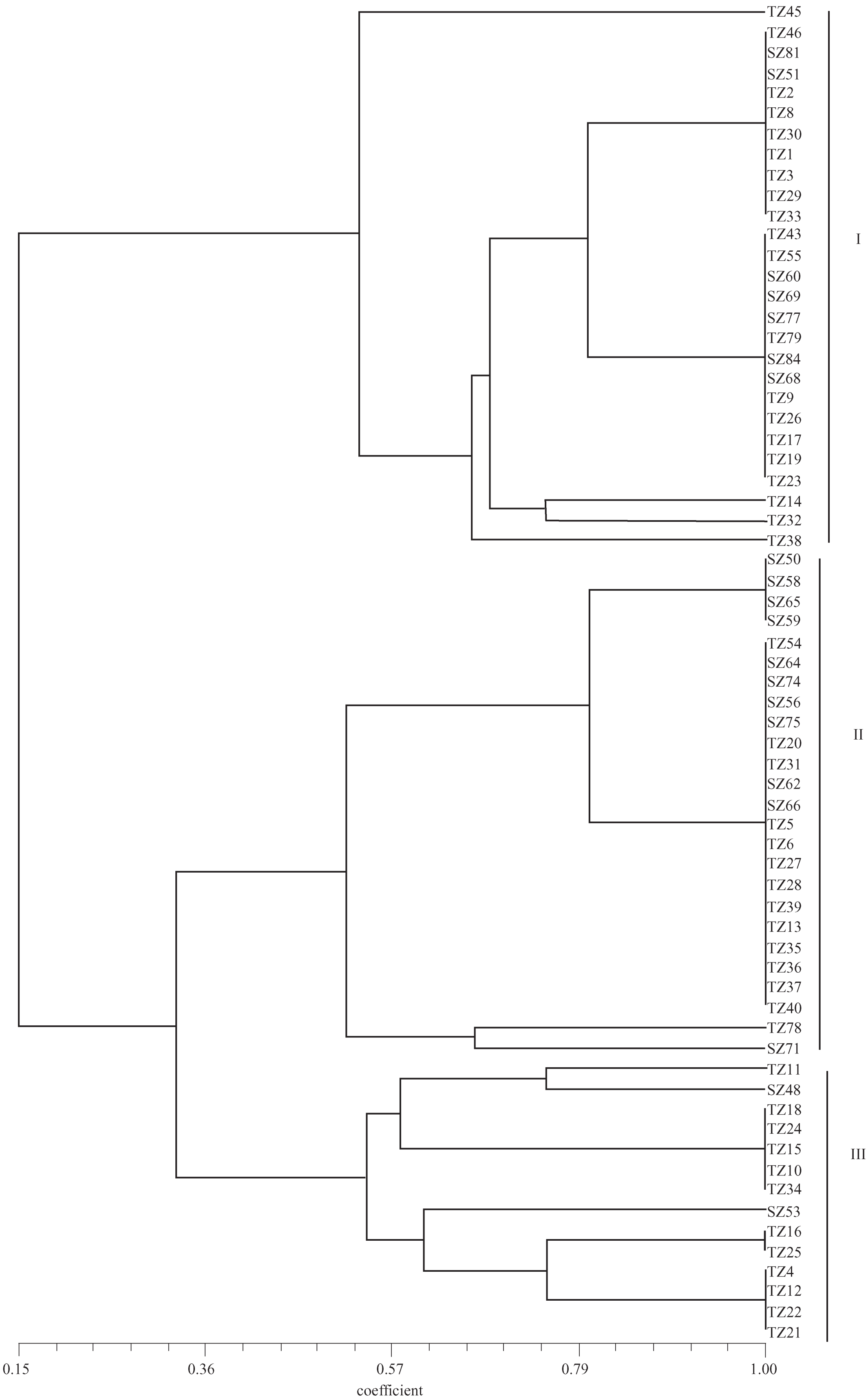

Fig. 3 Dendrogram based on SGP polymorphism

Bands SGP2, SGP6 and SGP8 occurred with much higher frequency in the Sichuan population than those in the Tibet population, while SGP5 and SGP9 were on the contrary (Table 2). There were a total of 12 patterns of bands identified in the Tibet population and 7 types in the Sichuan population. However, only 3 types were common, which represented a total of 42 accessions, 27 from Tibet and 15 from Sichuan (Table 3). The Shannon index of Tibet and Sichuan populations were 0.43 and 0.42, respectively, indicating they had a similar degree of genetic diversity.

Table 4 Number of bands for SGPs per accessions in naked barley studied

No. of bands	No. of samples	frequency of the population/%
2	7	10.61
3	8	12.12
4	22	33.33
5	29	43.94

3.3 Cluster analysis

Sixty-six accessions studied were clustered into 3 groups on the basis of the composition of the major SGPs, designated I, II and III (Fig. 3). Twenty of the Tibet and seven of the Sichuan accessions were divided into Group I, with 14 of the Tibet and 11 of the Sichuan accessions into II, and 12 of the Tibet and 2 of the Sichuan accessions into III, indicating the majority of the Tibet accessions were present in Group I and the Sichuan accessions were mainly located into Group II (Table 5). These findings also suggested the characteristics of major SGPs in the studied naked barley that are related to the collecting regions.

Table 5 Result of cluster analysis based on SGPs polymorphism

group	Tibet	Sichuan	Spring	Winter	total
I	20	7	23	4	27
II	14	11	20	5	25
III	12	2	13	1	14
total	46	20	56	10	66

3.4 Analysis of starch variation among the naked barley groups with different SGP patterns

The variations of those accessions of patterns 2, 3, 7, 8, 11 and 13 were carried out. The mean of starch content ranged from 50.59 to 56.54 and that of pattern 3 was significantly lower than that of pattern 2 and pattern 8. At the same time, the variation analysis revealed that the mean of the amylose content among different SGP patterns varied from 22.12 to 28.23 and that of pattern 7 and pattern 11 were the lowest and the highest, respectively, pattern 7 being significantly lower than pattern 11 (Table 6).

4 Discussion

Starch is the major component in barley grains and has an important impact on the quality and utilization of barley. All types of barley with specific starch properties can widen the range of usage and can promote the economic value of the crop. The resources of barley, especially the naked barley, are very rich in Qinghai-Tibet Plateau, China, which is beneficial to the development of the barley varieties with specific starch properties. Our study analyzed, for the first time, the SGP differences in the naked barleys from Qinghai-Tibet Plateau in China. Ten major SGP bands and 16 types of combination were found in the 66 naked accessions studied, and the frequencies of each band and pattern were greatly different, indicating that the variation of SGPs of naked barley in Qinghai-Tibet Plateau, China, was significantly marked.

Although no specific SGP bands with relation to the sampling sites were found, specific types of patterns existed, except for only 3 common ones, in the Tibet and the Sichuan populations. Meanwhile, in the cluster analysis, the majority of the Tibet accessions were located in Group I rather than Group II which was largely composed of Sichuan accessions. These indicated the SGP composition was significantly different between the Tibet and Sichuan populations, which may be

Table 6 Significance test of starch content among types of patterns

types of patterns	number	starch			amylose		
		mean	Range	significance	mean	range	significance
2	8	56.54 ± 3.01	53.58–61.59	bc	26.22 ± 1.92	22.42–27.89	ab
3	12	50.68 ± 8.04	37.36–60.43	a	24.55 ± 4.80	16.66–31.82	ab
7	4	50.59 ± 4.22	46.87–56.66	abc	22.12 ± 3.42	18.29–26.22	a
8	19	54.58 ± 3.95	47.37–61.35	b	25.11 ± 3.81	16.55–32.40	ab
11	4	54.31 ± 2.09	52.66–57.00	abc	28.23 ± 3.77	22.59–30.41	b
13	5	55.11 ± 3.99	49.42–58.66	abc	24.74 ± 2.22	21.95–27.69	ab
total	52	53.71 ± 5.29	37.36–61.59	–	25.12 ± 3.77	16.55–32.40	–

Note: Values with similar letters in the same column do not differ significantly ($P < 0.05$).

attributed to ecological factors and human activities at the collecting sites.

A number of studies have reported that some starch granule proteins have the activities of starch synthases. Patterns 2, 3, 7, 8 and 11 of this study showed significant variations in both starch and amylose content, suggesting these SGP bands might correspond to some starch synthases. Further studies are needed to verify the exact functions of these SGPs in the process of starch biosynthesis. At the same time, the accessions with different starch properties and SGP patterns were very useful for further studies and creation of novel starch varieties.

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