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ORIGINAL ARTICLE

Seasonal changes of endogenous soluble sugar and starch in different developmental stages of *Fargesia yunnanensis*

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Abstract The endogenous soluble sugars and starch are the main carbohydrates accumulated in bamboo culms and the seasonal changes of their contents are also related to the bamboo phenology. In this study, it is observed that the dormancy phase of new *Fargesia yunnanensis* culms continued as long as 6 months from November to next May. The time interval for the sugar storage between the branching and leafing stage and shooting stage is relatively short. Moreover, the branching and leafing stage overlapped the shoot buds formation stage. Starch granules were depleted when the branching and leafing stage commenced, but then were accumulated before the shooting stage. The endogenous soluble sugar content in 2- and 3-year-old culms decreased during the branching and leafing stage, but increased before the shooting stage, and then decreased after the shooting stage. In shoots, the endogenous soluble sugar and starch contents increased and decreased with culm elongation, respectively. Starch granules were accumulated more in nodes than in internodes in un-elongated internodes. Shoot buds and shoots are the main sink tissues for sugar storage in the whole clumps. The bamboo clumps should be fertilized and irrigated before and after the branching and leafing stage and shooting stage. The late autumn is the best season for the culm harvest.

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Introduction

Soluble sugars and starch are the principal forms of carbohydrate storage in vegetative tissues of plants, such as the stem and leaf sheaths of cereal [1]. In wheat, a lot of studies were involved in the dynamic changes of starch and soluble sugar levels in different vegetative sink tissues at different developmental phases [1–4]. However, few similar studies have been conducted in bamboos.

Bamboo consists of two general types: sympodial and monopodial. The sympodial bamboos are typically of tropical and subtropical origin and, therefore, cannot withstand freezing temperatures [5]. Monopodial bamboos usually shoot out of ground and then finish their branching and leafing in 2–3 months in spring, whereas the sympodial bamboos, e.g., *Fargesia* bamboos produce shoots in autumn. Therefore, there should be significant differences for the sugar and starch storage mechanism between monopodial and sympodial bamboos.

In monopodial bamboos, only moso bamboo (*Phyllostachys pubescens*) has been measured for the seasonal and height-dependent fluctuation of starch and free glucose contents so far [6]. However, few studies were involved in sympodial bamboos, especially for *Fargesia* bamboos. Many *Fargesia* species can survive in warm temperate zone and bear occasional low temperature in winter and, therefore, their sugar and starch storage mechanism is notable. Bamboo phenology underlies a range of management, conservation, and research issues [7]. Meanwhile, few studies are involved in the operation and management of bamboo forest, according to the changes of carbohydrates and moisture contents in culms with ages and seasons.

In this study, the endogenous soluble sugar, starch, and moisture contents of culms and shoots of *F. yunnanensis* in different seasons were determined, to understand the sugar storage mechanism and provide theoretical basis for bamboo forests harvest and management.

Materials and methods

Materials and phenology assessment

Bamboo culms of *F. yunnanensis* at different ages for this study were sampled from the bamboo Garden of Southwest Forestry University in Yunnan Province, China. The local rainy season begins from June to October and the rest months belong to dry season. The mean temperature and precipitation are 9.7 °C and 27 mm in winter, 16.2 °C and 44.2 mm in spring, 19.5 °C and 197.4 mm in summer, and 16.8 °C and 104.6 mm in autumn, respectively. At the study site, four bamboo clumps were selected to record the phenological changes with seasons from January to November 2013.

The culm age was determined by monitoring the growth starting from new shoots emerged from the ground. The 1, 2- and 3-year culms began to be cut in January 2013, which were actually only 4, 16 and 28 months old, respectively (Table 1). Three culms of each age class were harvested at each phenological stage, which were divided into three portions from culm base to top, i.e., 1st for bottom, 8th for middle and 15th for top, according to the culm heights and internode lengths. The culms of *F. yunnanensis* had 26–27 internodes and were about 10–11 m high. The 8th internode was usually the longest internode, which was identified as the middle portion. However, as the length of the 15th internode was half of the 8th internode, the 15th internode was then considered as the top. As for other shorter internodes in the top, they were too short and unsuitable for the experiment.

In addition, the shoot buds, the 1st internode (fast elongating, labeled as Internode 1), 3rd (elongation starting, labeled as Internode 3) and 5th (elongation unstarting, labeled as Internode 5) above ground of 0.5-month-old shoot, and the 1st internode (elongation completed and maturing, labeled as M1) of 1-month-old culms were obtained in May, September and October, 2013, respectively. The growth performance of individual internodes decreased with shoots height. Each internode was divided into bottom, middle and top from nodal bridge to sheath scar.

For the determination of endogenous soluble sugar and starch contents, samples from each culm were cut into small strips with a sharp razor and frozen immediately in liquid nitrogen. Other samples from culms or shoots were fixed in FAA fixative (1.85 % Formaldehyde, 45 % Alcohol and 0.25 % Acetic acid) for the starch localization. All samples were measured for the fresh weight in wild. The endogenous soluble sugar, starch and moisture contents were expressed based on fresh weight. The determination of each index was conducted in triplicate for each culm sample. Finally, the mean value of each index was calculated based on nine samples from three representative culms at each age class.

Endogenous soluble sugar content determination

Endogenous soluble sugar content was determined based on the method of phenol–sulfuric acid [8]. 0.2 g samples were ground to powder in a mortar and pestle using liquid nitrogen, and then were extracted with deionized water. The supernatants collected by centrifugation at 6000 rpm for 15 min were treated with 5 % phenol and 98 % sulfuric acid for 1 h. The absorbance at 485 nm was determined by spectrophotometer (752 N Hengping).

Starch content determination

Starch content was determined using the method of phenol–sulfuric acid [8]. The sediment from extract by centrifugation was dried, weighed and boiled with deionized water. The supernatants were used for the determination of starch content.

Moisture content determination

Moisture content was determined using the method of oven-dry test [9]. The moisture content was calculated through the difference values between the initial fresh weight and the oven-dry weight of samples. The samples were dried in an oven at 103 °C for the determination of oven-dry weight.

Starch granule localization

After the fixative in FAA, culm samples of different ages were dehydrated in a graded series of alcohol and then cut into sections, followed by the observation with a scanning electron microscope (Hitachi S-3000N).

As for the shoot and shoot bud samples, the periodic acid-Schiff (PAS) reaction was employed to observe the distribution of starch granules. After the fixative in FAA, transverse and longitudinal sections (7 μm) were cut using a rotary microtome and soaked in 0.5 % KIO_4 for 10 min, followed by 30 min in Schiff's reagent [10], and then were dehydrated in a graded series of ethanol and stained with

Table 1 The phenological period and sampling time of *F. yunnanensis*

Sampling time	Season	Germinating time	Age (months)	Phenology of bamboo clump
01, 2013	Winter/dry season (dormant stage)	09, 2012	4 (1-year-old culm)	New culms enclosed with culm sheaths with no branches and leaves were dormant from November until the end of next April
		09, 2011	16 (2-year-old culm)	
		09, 2010	28 (3-year-old culm)	
05, 2013	Spring/dry season (branching and leafing stage)	09, 2012	8 (1-year-old culm)	New culms began branching and leafing from May to the middle of June. Shoot buds began to form at the same stage
		09, 2011	20 (2-year-old culm)	
		09, 2010	32 (3-year-old culm)	
		–	0 (Shoot buds in soil)	
08, 2013	Summer/rainy season (before shooting)	09, 2012	11 (1-year-old culm)	The shoot buds continued forming and developing until the middle of September. A few shoots began growing out of the ground
		09, 2011	23 (2-year-old culm)	
		09, 2010	35 (3-year-old culm)	
10, 2013	Summer/rainy season (shooting stage)	09, 2013	0.5 (Shoots)	Most of shoots grew out of the ground constantly from the middle of September to the middle of October
		10, 2013	1 (Young culm)	The 1st internodes of most shoots had stopped their elongation in the middle of October
11, 2013	Autumn/dry season (after shooting)	09, 2012	14 (1-year-old culm)	Shooting stage was completed and most shoots had finished their height growth and went into dormancy until May of next year
		09, 2011	26 (2-year-old culm)	
		09, 2010	38 (3-year-old culm)	

Fast green FCF (For Coloring Food, Ameresco 0689) (Fast green 1 g + clove oil 100 ml + 100 % ethanol 100 ml). The sections were mounted in Canada balsam.

The paraffin sections were observed with a video camera linked to a converted fluorescence microscope (Nikon E400) and a Lenovo computer. The mean values derived from the experiments were compared by multiple comparisons using the least significant difference method (LSD).

Result

Phenology of *F. yunnanensis* with seasons

The phenological changes of *F. yunnanensis* have been recorded since January, 2013 (Table 1). The new culms were dormant enclosed with sheaths but no branches and leaves from November, 2012 to May, 2013. New culms sprouted new branches and leaves in top portions and a few in middle portions in spring. The branching and leafing stage usually commenced in the middle of May and ceased in the late of June. During this period, their shoot buds also formed from the base of 1- and 2-year old culms, especially more from the base of 1-year-old culms but few formed from the base of 3-year-old culms. The branching and leafing stage was prior to the onset of wet season.

In late August (rainy season), a few shoots began to germinate out of the ground in each bamboo clump. In September, most shoots sprouted and the bamboo clumps began to enter into shooting stage. Most shoots grew up to their maximum size before the end of the rainy season (November). The new shoots emerged by the end of the rainy season or the beginning of the dry season usually ceased the elongation and died. The branching and leafing stage and shooting stage were about 1 and 2 months, respectively, and the time interval between the two stages was very short (no more than 3 months) for the development of shoot buds and the photoassimilate storage.

Endogenous soluble sugar content of culms with seasons and portions

In winter (January), the new germinated culms enclosed with culm sheaths went dormant (Table 1). The endogenous soluble sugar content in 1-year-old culms (4-month) decreased with height, with the lowest mean value in top portions (Table 2). While in both 2- and 3-year-old culms, the opposite tendency was observed, with the highest value in the top portions. Meanwhile, it could also be observed that the endogenous soluble sugar content increased with age, with the highest value in 3-year-old culms, which implicated the available photosynthesis in 2- and 3-year-old bamboos in winter (Fig. 1a).

In spring (May), new culms began branching and leafing, which continued until the mid-June (Table 1). At this time, the endogenous soluble sugar content decreased with height at all age classes, with the highest value in the

Table 2 The content of starch and soluble sugar in different parts of *F. yunnanensis* at different seasons (%)

Age (years)	Portions	January (winter)		May (spring)		August (summer)		November (autumn)	
		Soluble sugar	Starch	Soluble sugar	Starch	Soluble sugar	Starch	Soluble sugar	Starch
1	Top	0.284 ± 0.000a	0.221 ± 0.017b	0.461 ± 0.002a	0.000	0.589 ± 0.008c	0.000	0.944 ± 0.002b	0.000
	Middle	0.374 ± 0.005b	0.252 ± 0.029b	0.491 ± 0.001b	0.000	0.431 ± 0.009b	0.000	0.564 ± 0.002a	0.000
	Bottom	0.406 ± 0.003c	0.140 ± 0.012a	0.526 ± 0.007c	0.000	0.304 ± 0.003a	0.000	0.451 ± 0.001a	0.000
2	Top	0.708 ± 0.001c	0.269 ± 0.006c	0.346 ± 0.001a	0.000	0.983 ± 0.001c	0.000	0.724 ± 0.004c	0.000
	Middle	0.681 ± 0.002b	0.310 ± 0.017b	0.473 ± 0.007b	0.000	0.737 ± 0.015b	0.000	0.427 ± 0.013b	0.000
	Bottom	0.539 ± 0.016a	0.151 ± 0.000a	0.501 ± 0.010c	0.000	0.634 ± 0.015a	0.000	0.383 ± 0.004a	0.000
3	Top	0.841 ± 0.002b	0.433 ± 0.002b	0.354 ± 0.002a	0.000	0.871 ± 0.007c	0.000	0.640 ± 0.012c	0.000
	Middle	0.630 ± 0.004a	0.214 ± 0.001a	0.371 ± 0.001ab	0.000	0.617 ± 0.188b	0.000	0.439 ± 0.006b	0.000
	Bottom	0.682 ± 0.005a	0.199 ± 0.015a	0.418 ± 0.005b	0.000	0.650 ± 0.004a	0.000	0.348 ± 0.003a	0.000

Means followed by the same letter in the same column are not significantly different at 0.05 probabilities. Values presented are mean ± standard deviation

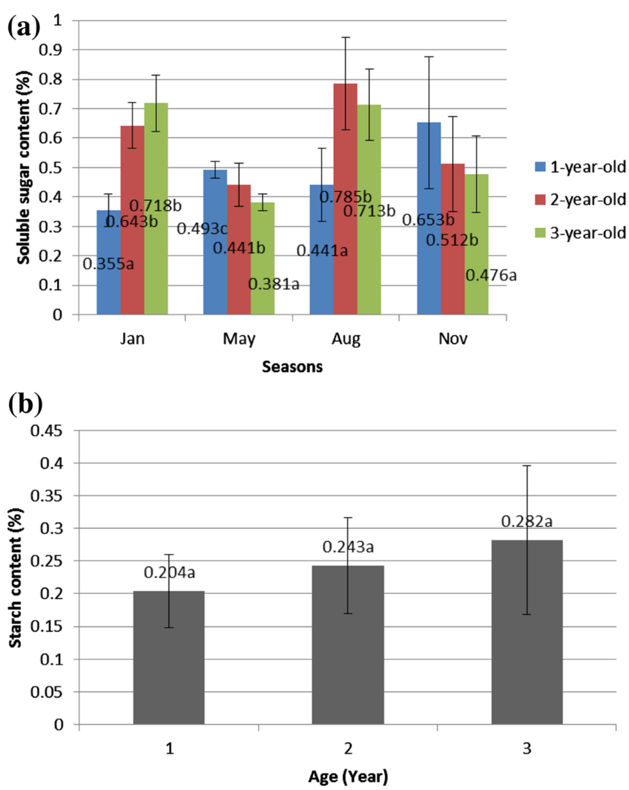


Fig. 1 Endogenous soluble sugar and starch contents in culms of different age. **a** The dynamic changes of soluble sugar contents in culms of different age with seasons. **b** The starch contents in culms of different age in winter, showing a slight increase with age. Means followed by the same letter are not significantly different at 0.05 probabilities

bottom portions of culms (Table 2). Meanwhile, the endogenous soluble sugar content also decreased significantly with age, with the highest mean value (0.493 ± 0.028 %) in 1-year-old culms (Fig. 1a).

In summer (August, local rainy season), most shoot buds of *F. yunnanensis* had formed and began shooting (Table 1). It could also be observed that the endogenous soluble content decreased significantly with height in culms at all age classes (Table 2). Besides, the endogenous soluble sugar content was significantly lower in 1-year-old culms than in 2- and 3-year-old culms (Fig. 1a).

In autumn (November, local dry season), all shoots had finished their height growth and the endogenous soluble sugar content was the highest in the top portions of culms at all age classes. However, by this time the endogenous soluble sugar content significantly decreased with age, with the highest mean value in 1-year-old (14-month-old) culms, showing that the 1-year-old bamboo began contributing carbohydrates most to the bamboo clump, but the contribution of 3-year-old culms was decreasing (Fig. 1a).

In general, the endogenous soluble sugar content in 1-year-old culms was higher in May and November than in January and August (Fig. 1a). However, a different trend could be observed in 2- and 3-year-old culms that the endogenous soluble sugar content in January and August was higher.

Starch content and localization with seasons

The starch content could be measured only in winter, while in other seasons it was undetected (Table 2). It could also be noted that the starch content was higher in the top and middle portions than in the bottom ones at all age classes, and meanwhile the starch content increased slightly with age, with the highest value in the 3-year-old culms (Fig. 1b), which is similar to the trend of endogenous soluble sugar content in culms of different ages.

With the scanning electron microscope (SEM), starch granules were easier to be observed in the parenchyma cells of the 3-year-old culms than those in culms of other age classes in January (Fig. 2a–c). In May, August and November, almost no starch could be determined in culms

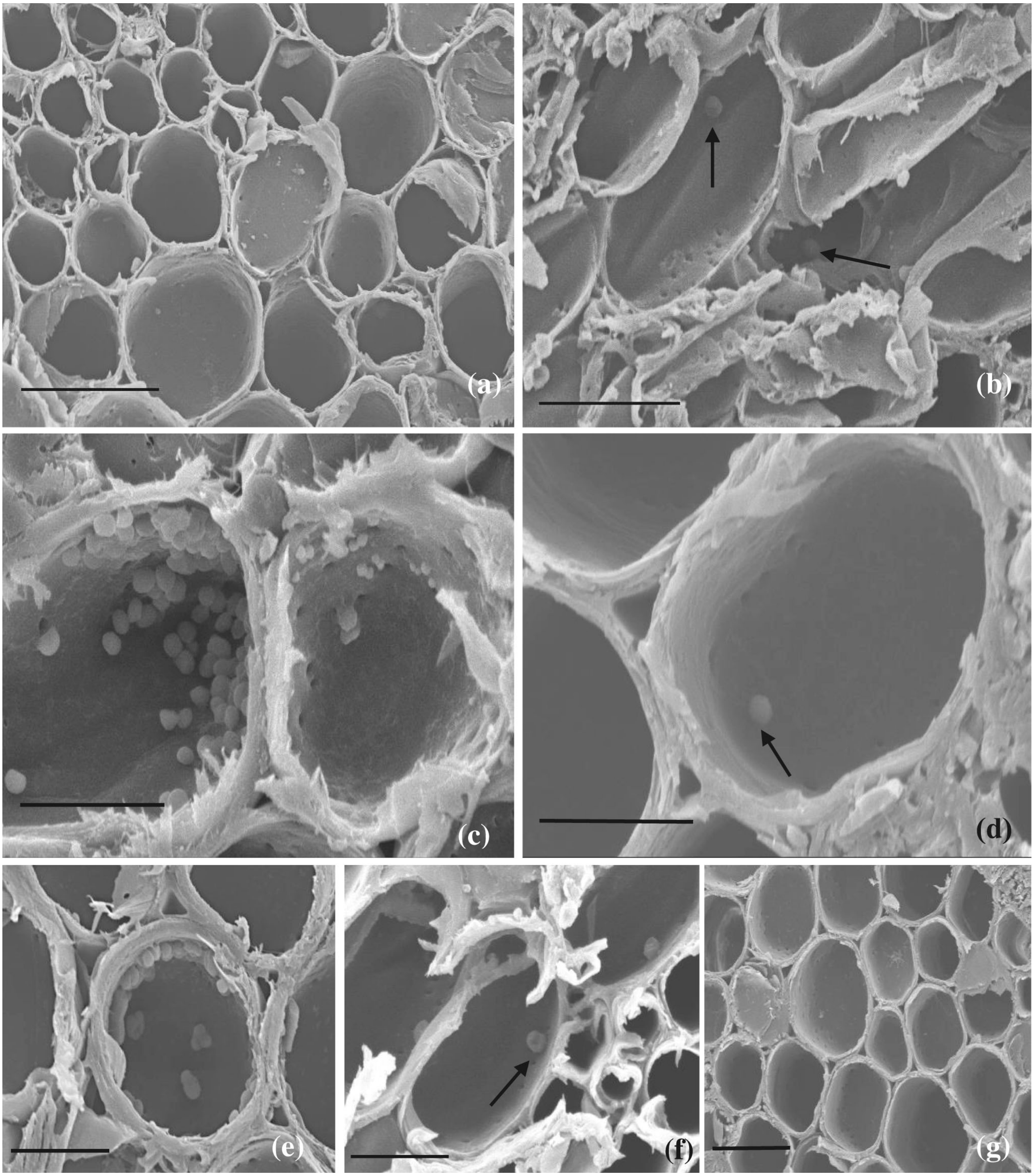


Fig. 2 Starch granule distribution in culms of *F. yunnanensis* in different months under SEM. **a** Parenchyma cells in the *top portion* of 1-year-old culms showing few starch granules in January. *Bar* = 50 μm. **b** A few starch granules (*arrow*) in the *top portion* of 2-year-old culms in January. *Bar* = 30 μm. **c** More starch granules in the *top portion* of 3-year-old culms in January. *Bar* = 20 μm. **d** Only a few starch granules (*arrow*) observed in the *top portion* of 3-year-old culms in May. *Bar* = 20 μm. **e** Starch granules observed in some parenchyma cells of the *top portion* of 3-year-old culms in August. *Bar* = 20 μm. **f** A few starch granules (*arrow*) observed in the top portion of 1-year-old culms in August. *Bar* = 20 μm. **g** Few starch granules observed in culms of all ages in November (here is the top portion of 3-year-old culms). *Bar* = 50 μm

Table 3 The moisture content in different parts of *F. yunnanensis* at different ages (%)

Age (years)	Portions	January	May	August	November
1	Top	67.517 ± 2.528a	46.879 ± 2.337a	38.927 ± 1.386a	36.677 ± 0.898a
	Middle	70.016 ± 3.454a	54.969 ± 0.239b	45.557 ± 1.040b	46.182 ± 0.113b
	Bottom	70.161 ± 2.616a	60.343 ± 0.026c	50.848 ± 1.124c	51.346 ± 1.330c
	Means	69.231 ± 3.835B	57.656 ± 2.687B	45.110 ± 5.373B	44.735 ± 6.544A
2	Top	45.059 ± 1.286a	33.889 ± 0.644a	27.303 ± 0.700a	35.035 ± 2.658a
	Middle	52.348 ± 2.662b	42.152 ± 2.234b	37.301 ± 2.088b	45.480 ± 2.510b
	Bottom	55.633 ± 0.041b	46.443 ± 0.064c	41.999 ± 1.353c	48.046 ± 2.124b
	Means	51.013 ± 5.082A	44.298 ± 2.145A	35.533 ± 6.759A	42.854 ± 6.685A
3	Top	32.340 ± 0.324a	36.350 ± 3.487a	32.000 ± 0.674a	31.840 ± 1.210a
	Middle	54.678 ± 3.483b	42.334 ± 4.153b	41.317 ± 0.829b	43.782 ± 0.664b
	Bottom	51.221 ± 0.319b	48.134 ± 1.860c	46.553 ± 1.773c	49.265 ± 2.230c
	Means	46.080 ± 10.664A	45.234 ± 2.900A	35.956 ± 6.539A	41.629 ± 7.949A

Means followed by the same letter in the same column are not significantly different at 0.05 probabilities. Values presented are mean ± standard deviation

at all age classes (Table 2). Actually, a few starch granules were still observed in some parenchyma cells of 3-year-old culms in the branching and leafing stage (in May) (Fig. 2d). However, due to their lower content, the starch granules were difficult to be determined. When the shoot buds formed and the shoots began to germinate, a few starch granules could not only be observed in 3-year-old culms (Fig. 2e), but also in 1-year-old culms, which had finished their branching and leafing (Fig. 2f). Meanwhile, the starch content was still below measurable limits in all portions. After the shooting stage (November), all new shoots had grown to their maximum size and no starch granules could be observed in culms of all age classes (Fig. 2g).

Moisture content in culms with seasons and ages

The moisture content in culms of each age class showed decreasing trend with height in all seasons (Table 3). In general, the moisture content also decreased with age, with the highest values observed in 1-year-old culms. It can also be noted that the differences between 2- and 3-year-old culms were not significant in all seasons, and even in May and August the moisture content of 2-year-old culms was slightly lower than that of 3-year-old culms. In addition, the moisture content in culms of each age class showed the similar trend that decreased firstly from January to August and then increased in November.

Starch localization and endogenous soluble sugar content in shoots and shoot buds

During and after the branching and leafing stage of 1-year-old culms, most shoot buds of the bamboo clump also formed. By PAS reaction, it could be observed that there were lots of starch granules in the undifferentiated internodes and parenchyma cells between vascular bundles in shoot buds (Fig. 3a, b) Meanwhile, there were more starch granules distributing in the parenchyma cells close to the culm skin (Fig. 3c). It could also be noted that most starch granules distributed around the nuclei or close to the cell wall in parenchyma cells. However, there were only one or two starch granules in each parenchyma cell in the internode close to the pseudorhizome (Fig. 3d) and few starch granules in pseudorhizome (Fig. 3e), which indicated that the shoot buds are the sink tissues for the starch storage.

In 2-week-old shoots, the 1st internode above ground (internode 1) was fast elongating, the elongation of the 3rd internode (internode 3) also initiated and the 5th internode (internode 5) was still un-elongated. Table 4 shows that the top portions had higher content of endogenous soluble sugar than the bottom portions in the elongating internodes (internode 1 and 3). However, in the un-elongated internodes (internode 5), no significant trend of endogenous soluble sugar content was observed between different portions. Starch contents also showed similar trend in shoots. This phenomenon was caused by the endogenous sugar and starch consumption of the intercalary meristem in bottom portions of internode. Besides, it could also be noted that the endogenous soluble sugar content decreased with height, while the starch content increased with height. Meanwhile, the endogenous soluble sugar content was observed to be lower than the starch content in the un-elongated internodes (internode 5), but higher in the fast elongating internodes (internode 1), which indicated that the endogenous soluble sugar was consumed for the starch synthesis in un-elongated internodes and the starch was hydrolyzed for the sugar consumption in internode elongation. In internode 3, the soluble sugar content was still lower than the starch content, which was because the

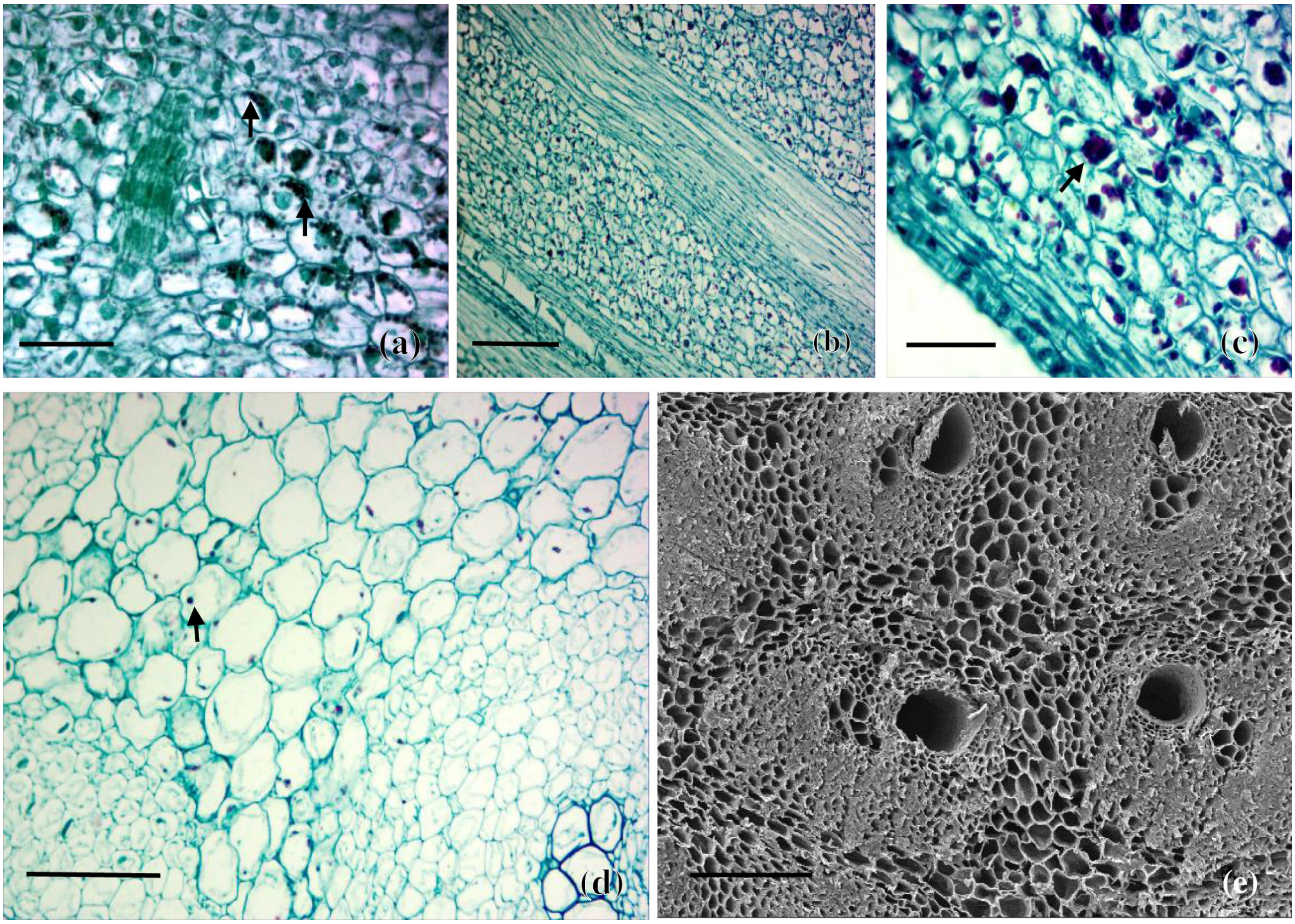


Fig. 3 Distribution of starch granules in shoot buds of *F. yunnanensis* in May. Light (**a**-**d**) and SEM (**e**) micrographs. **a** The longitudinal section of the undifferentiated internode in the top portions of shoot buds, showing the distribution of starch granules close to cell walls (*arrows*). *Bar* = 10 μm. **b** Abundant of starch granules in parenchyma cells between vascular bundles in longitudinal section. *Bar* = 40 μm. **c** The longitudinal section of parenchyma cells close to the culm skin, showing abundant of starch granules around the nuclei (*arrow*). *Bar* = 10 μm. **d** The transverse section of the internode in shoot bud close to pseudorhizome, showing one or two starch granules close to cell walls in each parenchyma cells (*arrow*). *Bar* = 20 μm. **e** The transverse section of the internode in pseudorhizome. Few starch granules could be observed. *Bar* = 200 μm

elongation of this internode was just initiated and the sugar consumption was relatively small as compared to that of the fast elongating internode (internode 1). Hence, the endogenous soluble sugar and starch contents were lower and higher in Internode 3 than in Internode 1, respectively. In the 1st internode of 1-month-old shoots (M1), the endogenous soluble sugar and starch contents in top portions were higher than that in bottom ones, and meanwhile the endogenous soluble sugar content was also higher than the starch content.

Figure 3 also illustrates that more starch granules accumulated in the un-elongated internode (top internode and internode 5) than in the elongating (internode 3 and 1) and elongated internodes (M1). With internode elongation, the number of starch granules decreased in parenchyma cells. In the un-elongated internodes, there were more starch granules in the nodes than that in the internodes (Fig. 4a), many of which were found around the nuclei (Fig. 4b). In the un-elongated internode (internode 5), lots of starch granules were observed in the parenchyma cells between vascular bundles (Fig. 4c). Once the elongation of internodes initiated, the number of starch granules decreased significantly (Fig. 4c–e), and a small number of starch granules could still be observed in the parenchyma cells close to the phloem and metaxylem, but no starch granules could be observed in the parenchyma cells between vascular bundles. In the 1-month-old shoots, the 1st internodes had completed their elongation and only a few starch granules could be observed close to cell walls in long cells (Fig. 4f). Generally, the content of endogenous soluble sugar was higher than that of the starch in the elongating and elongated internodes. Meanwhile, the endogenous soluble sugar content increased, but the starch content decreased with internode elongation.

Table 4 The soluble sugar content in different parts of bamboo shoots (%)

Internode	Portions	Soluble sugar	Starch
5	Top	0.476 ± 0.052a	0.670 ± 0.067a
	Middle	0.428 ± 0.039a	0.720 ± 0.082ab
	Bottom	0.490 ± 0.050a	0.880 ± 0.062b
	Means	0.465 ± 0.061A	0.756 ± 0.193B
3	Top	0.608 ± 0.052a	0.674 ± 0.104a
	Middle	0.526 ± 0.067a	0.583 ± 0.061a
	Bottom	0.526 ± 0.066a	0.534 ± 0.064a
	Means	0.553 ± 0.073B	0.597 ± 0.116A
1	Top	0.913 ± 0.105a	0.610 ± 0.079a
	Middle	0.828 ± 0.067a	0.519 ± 0.063a
	Bottom	0.731 ± 0.077a	0.548 ± 0.053a
	Means	0.824 ± 0.113C	0.553 ± 0.085A
M1	Top	1.114 ± 0.071a	0.682 ± 0.088b
	Middle	1.009 ± 0.057a	0.502 ± 0.065a
	Bottom	0.975 ± 0.075a	0.455 ± 0.063a
	Means	1.033 ± 0.095D	0.546 ± 0.109A

Means followed by the same letter in the same column are not significantly different at 0.05 probabilities. Values presented are mean ± standard deviation

Discussion

Phenology of *F. yunnanensis*

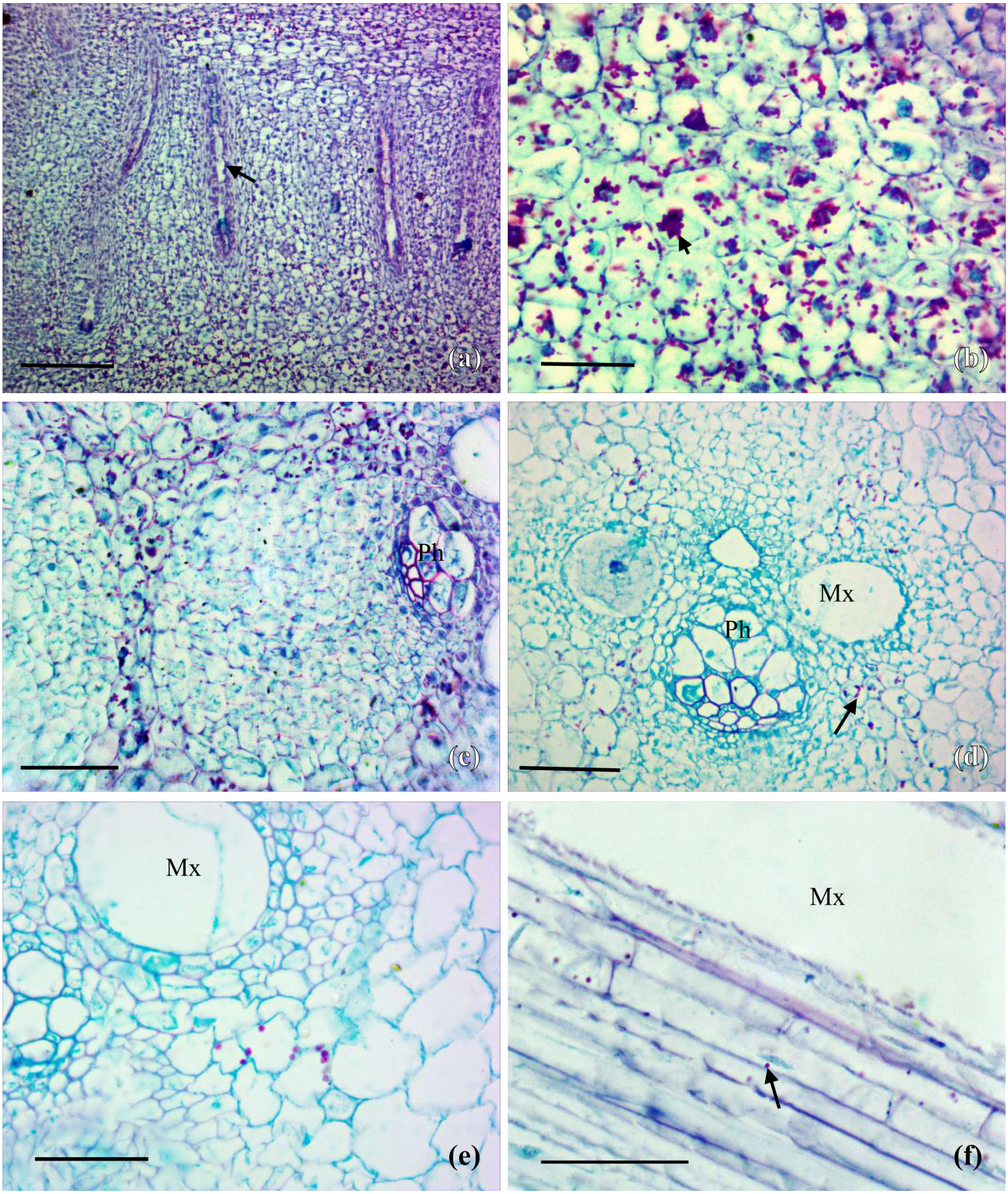
Vegetative phenology reflects the adaptation of graminoid processes to the giant, arborescent form, and the need to complete elongation of growth axes in a single growth period [7]. In *F. yunnanensis*, new culms were always dormant during dry season, which caused the overlap between the branching and leafing stage and shooting buds formation stage, and also caused the short time interval between the branching and leafing stage and the shooting stage for sugar storage in culms, which is significantly different from the phenology of *Ph. edulis* [11]. Both shooting stage and branching and leafing stage of most monopodial bamboo species occur in spring, so that they have a long phase (three seasons) for the sugar accumulation and the formation and development of shoot buds, while there is relatively short time (no more than 3 months) for the sugar accumulation and the development of shoot buds in *F. yunnanensis*. Therefore, differences in the sugar accumulation mechanism between different bamboo species lied on their different phenology. In addition, the branching and leafing stage in *F. yunnanensis* occurred at the end of the dry season and preceded the onset of rainy season, which was significantly different from that in *Bambusa arnbemica* [7]. The adaptive advantages of leaf flush prior to the onset of wet season rains are unclear, but may involve use of optimal conditions for photosynthesis and the avoidance of herbivory [12]. Most shoots of *F. yunnanensis* completed their height growth at the end of the rainy season, which is similar to that in *B. arnbemica* [7].

Endogenous soluble sugar and starch contents with seasons

Previous study has shown that the free sugar contents were generally lower in autumn and winter than in spring and summer in *Phyllostachys pubescens* [6]. However, the endogenous soluble sugar content in 2- and 3-year-old culms of *F. yunnanensis* was much lower in spring than in other seasons. As for starch content, it has been reported to be lower in summer than in winter and spring in *P. pubescens* culms [6]. In *F. yunnanensis*, the starch could be determined and observed only in winter, while in other seasons it was below measurable limit and could not be determined, which is different from that in *P. bambusoides* showing a maximum value of starch content in May [13]. This is caused by the phenological differences between different bamboo species. In *F. yunnanensis*, it is difficult to accumulate enough starch for determination, because of the short interval (2–3 months) between the branching and leafing stage and the shooting stage. Most photoassimilates were transported into shoot buds and shoots, so the starch content in culms of these stages was undetectable.

Starch accumulation in stem tissues occurs when carbohydrate supply is excess of demands and can act as temporary carbon storage, which can be reutilized [1]. In January, for 1-year-old culms of *F. yunnanensis* enclosed with sheaths but no branches and leaves, most carbohydrates came from their mother bamboos. By this time, the endogenous soluble sugar content increased with age with the highest values in 3-year-old culms, showing that 3-year-old culms can still supply enough photoassimilates to the bamboo clump. Meanwhile, the starch content increased with age, with the highest value in 3-year-old culms. Therefore, winter is not the appropriate time for bamboo forest harvest. Moreover, Okahisa et al. also considered that the perishable properties of bamboo are mainly caused by its high sugar and starch contents, which are foods for fungi or insects [6].

When in May, new culms of *F. yunnanensis* began branching and leafing, and old bamboos also sprouted new branches and leaves. New shoot buds also began to form at this time. Therefore, it would deplete a large amount of sugars and then almost all starches were depleted and reutilized. Therefore, the endogenous soluble sugar content in 1-year-old culms increased to achieve the highest values and decreased significantly in 2- and 3-year-old culms. Meanwhile, few starches could be determined in culms of all ages. It indicated that the sugar consumption was the


(a)
(b)
Ph
(c)
Mx
Ph
(d)
Mx
(e)
Mx
(f)

◀**Fig. 4** The distribution of starch granules in internodes with different developmental degree in 0.5- (**a**-**e**) and 1-month-old (**f**) shoots. Light micrographs. *Mx* Metaxylem, *Ph* pholem. **a** The longitudinal section of the internode in the top portion, showing more starch granules in node than in internode and the differentiation of vessels (*arrow*). *Bar* = 40 µm. **b** Lots of starch granules around the nuclei in node of top portion (*arrow*). *Bar* = 10 µm. **c** The transverse section of the 5th internode above ground (internode 5), showing abundant of starch granules in parenchyma cells around the fiber bundles. *Bar* = 20 µm. **d** The transverse section of the 3rd internode above ground (internode 3), showing the decrease of starch granules (*arrow*) in parenchyma cells between metaxylem and phloem. *Bar* = 20 µm. **e** The transverse section of the 1st internode above ground (internode 1), showing that only several starch granules were left close to the cell wall. *Bar* = 10 µm. **f** The longitudinal sections of the 1st internode of 1-month-old shoot (elongation completed and maturing, labeled as M1), showing that a few starch granules (*arrow*) could still be observed in those parenchyma cells close to metaxylem vessels. *Bar* = 10 µm

main characteristics of the whole bamboo clump during branching and leafing stage.

From May to August, the endogenous soluble sugar content was significantly lower in 1-year-old culms than in 2- and 3-year-old culms, which may be due to the fact that the 1-year-old culms produced more shoot buds than others and consumed more sugars. Although starch granules could still be observed in culms including the new culms under SEM, the starch content was still below the measurable limit. After all, either in the formation process of shoot buds or in the followed shooting stage, consuming considerable sugars was needed. Therefore, proper fertilization in May and August is necessary. When entering into August and November, the 2-year-old culms began to supply the most carbohydrates to the bamboo clump, based on the highest content of endogenous soluble sugar. Meanwhile, the contribution of 3-year-old bamboos to the bamboo grove was reducing.

In November, the shooting stage of *F. yunnanensis* completed and new shoots grew to their maximum size, which depleted the sugar storage of the bamboo clumps and directly caused the lower sugar content in 2- and 3-year-old culms than in 1-year-old culms. The high endogenous soluble sugar content in 1-year-old culms may benefit from their new branches and leaves and their sugar contribution to the clump increased. When the new culms entered into dormancy in winter (January), plenty of starches and endogenous soluble sugar are essential for the sugar storage in culms. Therefore, the fertilization for the clumps is important after the shooting stage. In general, *F. yunnanensis* forest should be fertilized for twice or 3 times in 1 year. Besides, previous reports have shown that the best season for the bamboo harvest is autumn, in which the free glucose and starch contents are low [6, 13]. In the present investigation, the optimum harvest time is late autumn, i.e., November, based on the low content of endogenous soluble sugar and starch in 3-year-old culms. In May, although the endogenous soluble sugar in culms is lower, harvest at this time will decrease the development and production of shoots.

Endogenous soluble sugar and starch contents with portions

In January, the trend of the endogenous soluble sugar content was shown in the order of top < middle < bottom in 1-year-old culms (4 months), while in 2- and 3-year-old culms it showed the opposite trend, i.e., top > middle > bottom, which was caused by the leaf photosynthesis of the 2- and 3-year-old culms. However, 1-year-old culms had no branches and leaves and their endogenous soluble sugar storage mainly depended on their mother bamboos, so they showed the trend of top < middle < bottom. The same trend in the culms of all ages in May is due to their extensive branching and leafing in top portions that consumed large amounts of sugar. When in August and November, all culms had sprouted plenty of branches and leaves in top portions and plenty of photoassimilates were produced and mainly supplied to the development and growth of shoot buds and shoots. Therefore, the trend of endogenous soluble sugar was shown as top > middle > bottom. The endogenous soluble sugar content in culms depended on the bamboo phenology.

As for starch, it has ever been reported to be higher in the top portions than in other portions [14]. While in *P. pubescens*, no special trend was observed [6]. In *F. yunnanensis*, the starch content was also higher in top and middle than in bottom.

Moisture content in culms with seasons and age

Regarding the moisture content in culms, numerous investigations have revealed that it is higher at the base than the top, which is due to the high amount of parenchyma present in the base [15, 16]. Similar trend was also observed in the present study.

In general, the moisture content in culms not only decreased with age, but also decreased with seasons, except in August. The lowest values of moisture content in 2- and 3-year-old culms in August implied that the bamboo clump needed to consume high amounts of water during shooting stage. After the shooting stage, the moisture content in 2- and 3-year-old culms was still far lower than that in winter. The knowledge of the water status in plant tissue may give an indication of the water requirement and the timing of irrigation [17]. Therefore, irrigation is essential before and after the shooting stage. In addition, the 3-year-old culms had the lowest moisture content by this time, which also showed that it is the optimum time for bamboo harvest.

Endogenous soluble sugar and starch contents with development of shoots

Abundant of starch granules accumulated in shoot buds, but in the pseudorhizome and the internode close to the pesudorhizome localized few starch granules. Meanwhile, plenty of starch grains could also be observed in the un-elongated internodes of shoots, which implied that shoot buds and shoots are the main sink tissues for starch storage and there is a starch pre-storage mechanism for the formation and fast growth of bamboo shoots.

With shoot development and internode elongation, the endogenous soluble sugar content increased, but there was a constant decrease in the starch content and the number of starch granules, which was mainly due to the hydrolysis and consumption of starch in elongating and elongated internodes. More and more starches were hydrolyzed into endogenous soluble sugar for the sugar consumption during internode elongation. Fischer and Höll considered that the increase in sugar concentration may be a result from the degradation of starch [18]. In maize, Mohammadkhani and Heidari reported similar trend and they considered that starch may play an important role in the accumulation of endogenous soluble sugars in cells [19]. The internode elongation process is accompanied by the hydrolysis and consumption process of starch in shoots.

Regarding the localization of starch granules in culm, it can be observed that most starch granules localized in the parenchyma cells, around nuclei or close to cell walls. As parenchyma cells are the storage tissue for the plant, abundant starch granules can fill up the cells [16]. Similar trend was also reported in wheat stems that the starch granules are found predominantly in parenchyma cells surrounding the vasculature [1].

Conclusion

The peculiarly seasonal changes of carbohydrates in culms of *F. yunnanensis* lie on its peculiarly vegetative phenology, which has a short period of 2–3 months for the sugar storage. The 3-year-old culms still play a vital role in the sugar accumulation for the whole clump in January due to their high content of endogenous soluble sugar. Shoot buds or shoots are the main sink tissues for the sugar storage. The endogenous soluble sugar and starch contents increased and decreased, respectively, with internode elongation. Starch granules are mainly located in the parenchyma cells around the nuclei or close to the cell walls. The late autumn is the best season to harvest bamboo for the low sugar and moisture contents. *F. yunnanensis* forest needs to be fertilized and irrigated before and after the branching and leafing stage and the shooting stage.

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