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Discovery of C_4 species at high altitude in Qinghai-Tibetan Plateau

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Abstract **Plant specimens are collected from the areas between latitude 27°42′N and 40°57′N, and longitude 88°93′E and 103°24′E, with an altitudinal range from 2210 to 5050 m above the sea level in Qinghai-Tibetan Plateau. The stable carbon isotope analysis indicates that two of Chenopodiaceae and six of Poaceae in the samples are C_4 plants. Four of the C_4 plants are found in 11 spots with altitudes above 3800 m, and *Pennisetum centrasiaticum*, *Arundinella yunnanensis* and *Orinus thoroldii* are present in six spots above 4000 m, even up to 4520 m. At low CO_2 partial pressure, that sufficient energy of high light improving C_4 plant's tolerance of low temperature and precipitations concentrating in growing season probably are favorable for C_4 plants growing at high altitude in Qinghai-Tibetan Plateau.**

Keywords: Qinghai-Tibetan Plateau, high altitude, C_4 plants.

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Three modes of photosynthesis predominate in terrestrial plants: the C_3 mode, employed by most higher plant species; the CAM mode, employed by about 20000 higher plant species; and the C_4 mode, employed by approximately 8000 higher plant species[1,2]. Although far fewer species use the C_4 pathway, because of substantial and potential significance for their ecological and economic significance, great attention has been paid to understanding physiological character and global distribution of C_4 plants[3].

Earlier investigations have shown that the abundance of C_4 species has a high correlation with the temperature[4]. C_4 plants dominate most low elevation landscapes in the tropics and subtropics[5], become rare in high latitude regions and absent from regions above 60°N[6,7]. As with latitudinal trends, the abundance of C_4 species decreases with altitude until C_4 plant disappears[8]. Generally, C_4 species drop out of the flora above 2000—3000 m[9—12], C_4 species above 4000 m have only been found in Kenya and northern Argentina[13,14].

In China, the lists of C_4 species edited by Yin et al.[15] have provided important fundamental data for encouraging further ecological and floristic assessments of C_4 distribution. However, the C_4 distribution with altitude and latitude in China, especially in Qinghai-Tibetan Plateau, is still little known. In this paper, we investigate C_4 species distribution in Qinghai-Tibetan Plateau and discuss what factors influence the C_4 species distribution at the high altitude.

1 Materials and methods

The Qinghai-Tibetan Plateau, known as "The roof of the world", with an average altitude of over 4000 m above sea level (masl), is the highest and largest plateau on the earth with unique environment and climate characteristics. The study covers a vast area from latitude 27°42′N to 40°57′N, and from longitudes 88°93′E to 103°24′E, with a large altitudinal range from 2210 to 5050 masl. The vegetation is characterized by a zonal pattern from the southeast to the northwest, followed by a decreasing gradient of moisture, ranging in forest, meadow, steppe, and desert steppe.

All species were collected from sites with a variety of vegetation types including forest, shrub, steppe, alpine meadow, and desert steppe. In the summer of 1999, total species were collected from 106 locations along the latitude from north to south, at an interval of approximately 10—50 km. Longitude, latitude, and altitude of the sites were measured using a GPS 12 (GARMIN).

Plant samples are identified at Northwest Institute of Plateau Biology, Chinese Academy of Sciences. C_4 plants are selected based on the list of C_4 plants[2,15—19], and stable carbon compositions were conducted on leaves of C_4 species, especially those from high altitude. 3—10 leaves (mostly 5) from at least three different adult individuals were collected. Leaves were cleaned, dried in oven at 60 ℃ for 24 h and ground to fine powder. Ground leaf samples (8—10 mg) were weighed into a glass tube with a short length (c. 2 mm) of silver wire (to remove any trace halogens which might interfere with the mass spectrometer results). The tube was evacuated, sealed and heated to 450℃ in a furnace, until combustion of the organic matter was complete. The CO_2 produced was passed to a mass spectrometer (MAT-252). The relative abundances of ^{12}C and ^{13}C were measured. The working standard was related to Pee Dee Belemnite (PDB). The internal reproducibility of the mass spectrometer was 0.02‰ and that of the working standard 0.05‰. Subsamples usually showed negligible deviations (mean s.d. was 0.2‰), but if significant differences occurred, two additional samples were analyzed to ascertain results.

The ratio of the two stable isotopes of carbon (^{13}C: ^{12}C) is conventionally expressed in per mil (‰) term as $\delta^{13}C$:

$$\delta^{13}C(‰) = [(R_{sample} - R_{control})/R_{control}] \times 1000,$$

where R_{sample} and $R_{control}$ are, respectively, the ^{13}C: ^{12}C ratios of the sample and the universally accepted PDB standard. The pretreatment and analysis of samples were conducted in Isotope Laboratory at Institute of Geology and Physical Geography of Chinese Academy of Sciences.

2 Results

The C_3 and C_4 photosynthetic pathways fractionate carbon isotopes to different degrees; C_3 and C_4 plants have $\delta^{13}C$ values ranging from about –22‰ to 30% and –10‰ to –14‰, respectively[20]. Carbon isotope analysis indicates that some plants are not C_4 plants and of course, these plants are excluded[21]. 8 species (Table 1) have been assigned as having C_4 photosynthetic pathway in our collected species. They occur within Chenopodiaceae and Poaceae. Two species of Chenopodianceae are *Atriplex centralasiatica* and *Salsola ruthenica*; six species of Poaceae are *Chloris virgata*, *Eragrostis ferruginea*, *Eragrostis nigra*, *Arundinella yunnanensis*, *Orinus thoroldii*, and *Pennisetum centrasiaticum*. The distribution of these species in Qinghai-Tibetan shows the following characters:

(i) Some C_4 plants distribute at high altitude in Qinghai-Tibetan Plateau (Table 1). Among the 106 spots

Table 1 Altitude, latitude, longitude, mean minimum growing season temperature (MMGST obtained by spatial interpolation based on a database of the MAT over the last 10 a from 38 weather stations in Tibet) and $\delta^{13}C$ of C_4 plants sampled in Qinghai-Tibetan Plateau

Family	Species	Altitude/m	Latitude (N)	Longitude (E)	MMGST/℃	$\delta^{13}C$ (‰)
Chenopodiaceae	*Atriplex centralasiatica* Il jin	3243	36°27′	98°14′	8.0	–12.70
	Salsola ruthenica Il jin	3370	39°17′	94°16′	7.0	–11.20
Poaceae	*Pennisetum centrasiaticum* Tzvel.	2210	36°46′	103°14′	13.1	
		3360	29°54′	93°33′	8.5	–11.84
		3420	35°54′	94°43′	7.3	
		3480	29°53′	93°18′	7.9	–11.99
		3590	29°24′	90°53′	7.5	
		3630	29°16′	90°28′	7.3	–12.54
		3700	29°47′	91°23′	6.8	–11.10
		3780	29°50′	91°44′	6.4	–12.07
		3780	29°21′	89°40′	6.5	
		3850	29°57′	92°51′	5.9	–12.10
		3850	29°20′	88°58′	6.2	
		3870	29°10′	89°02′	6.1	–13.03
		3900	29°46′	90°47′	5.8	–11.95
		4115	30°01′	90°38′	4.6	–11.41
		4230	28°37′	89°40′	4.2	–11.39
		4290	28°50′	89°53′	3.9	–11.92
		4520	28°26′	90°24′	2.7	–12.59
	Arundinella yunnanensis keng ex BS Sun & ZH Hu	3115	29°34′	94°29′	9.8	
		3150	29°45′	94°14′	9.6	
		3250	29°48′	93°50′	9.1	–11.42
		4170	29°52′	92°35′	4.3	–11.63
	Eragrostis ferruginea (Thunb.) Beauv.	2870	27°25′	88°56′	11.6	
		3150	29°44′	94°07′	9.6	–13.05
		3300	27°30′	88°56′	9.4	–11.28
	Eragrostis nigra Nees ex Steud.	3120	29°35′	94°29′	9.8	
		3420	35°54′	94°43′	7.3	
		3480	29°53′	93°18′	7.9	–11.48
		3630	29°16′	90°28′	7.3	
		3700	29°47′	91°23′	6.8	–12.57
	Orinus thoroldii (Stapf ex Hemsley) Bor, Kew. Bull.	3590	29°24′	90°53′	7.5	
		3705	29°20′	90°14′	6.9	
		3750	29°19′	89°53′	6.7	
		3780	29°21′	89°40′	6.5	
		3850	29°20′	88°58′	6.2	–12.61
		4335	30°06′	90°33′	3.4	–13.69
	Chloris virgata Swarbrick	3820	29°20′	89°14′	6.3	–13.00

in this study, C_4 plants are found in 31 sites, with 18 sites above 3500 m, 11 sites above 3800 m and 6 sites above 4000 m, even up to 4520 m.

(ii) Most of the C_4 species distribute in south Tibet (Table 1). All samples are from regions between 27°42′N and 39°28′N. The localities of 27 samples are concentrated between 27°42′N and 30°00′N and only four grow above 35°54′N.

(iii) The mean minimum growing season temperature is only 7.2℃ (Table 1). Of all the 31 spots with C_4 plants, there are only 2 sites with the temperature higher than 10℃, 8 sites between 8 and 10℃, 13 sites between 6 and 8℃, and 8 sites below 6℃.

(iv) Almost all of the C_4 plants are collected from steppe, and alpine meadow, while few from forest and desert steppe.

3 Discussion and conclusions

Research on C_4 plant ecology shows that the origin, evolvement and distribution of C_4 plant are related to temperature, light[4,22—24], precipitation[25—28], and in theory, CO_2 partial pressure[29].

The distributional pattern in C_4 species indicated that the abundance of C_4 plants is highly dependent on temperature[4,22—24]. In the tropics and subtropics, more than two thirds of all grasses are C_4[8]. More than 90% of the principal savanna grasses of low latitudes are C_4[5]. East Asia and eastern North America exhibit 30%—70% of C_4 species, and gradual relationships between latitude and C_4 occurrence[8]. C_4 species become uncommon in all regions above latitude of 40°—50°[8], and are rare above 60°N[6,7]. Geological records indicate that the abundance of Holocene C_4 species is more than that of the Last glacial in North America and Loess Plateau of China[30,31]. As with latitudinal trends, temperature indices are closely correlated with elevation trends and reveal a decrease in abundance of C_4 species with altitude[8－13]. Further research shows that the growing season temperature is closely correlated with abundance of C_4[9—11,32—35], but the winter temperatures do not suggest any clear correlation with abundance of C_4[36]. For example, C_4 species are rare in North America where the mean minimum temperature of the warmest month is below 8℃[22], and C_4 plants are absent in arid regions of central Asia where the mean minimum growth season temperature is lower than 6—8 ℃[33]. The fact that C_4 species concentrated in the south Tibet and generally decreased with the altitude indicates that temperature also plays an important role in controlling the distribution of C_4 plants in Qinghai-Tibetan Plateau.

However, in this study area the growing season temperature of C_4 plants is lower than other places in the world. The mean minimum growing season temperature of almost 68% sites with C_4 plants is below 8℃ and that of about 38% sites above 4000 m among them is below 6℃. The lowest temperature even reaches 2.7℃(Table 1), indicating that some special climate conditions in Qinghai-Tibetan Plateau such as high light and wet summer are probably favorable for C_4 plants.

The high light in Qinghai-Tibetan Plateau probably plays an important role in C_4 plant growth. The contribution of light to C_4 concerns tow aspects: (i) At low CO_2/O_2 ratios, C_3 plants are disadvantage relative to C_4 plants. However, C_4 plants can achieve a relatively high quantum yield by suppressing photorespiration at low CO_2/O_2 ratios[1]. (ii) The model predicts that the C_4 plant tolerance of low temperature is improved with the CO_2 partial pressure decreasing so that C_4 plant can develop under low temperature[29]. However, both need extra energy in order to achieve a relatively high quantum yield, to endure low temperature. Especially, at low temperature, much more light energy is required to assimilate CO_2 in C_4 plant[37]. The high light and long sunlight make the total radiation of Tibetan Plateau reach about 117.23 $kJ/m^2 \cdot month$[38]. As a result, this provides enough energy in the C_4 dicarboxylate cycle and assimilating CO_2 at low CO_2/O_2 ratios, and compensates the energy needed for photosynthesis at low temperature and finally, probably leads C_4 plants to improve efficiency of carbon-fixation and endurance to low temperature at a high altitude.

In addition to the index of growing season temperature, the growing seasonal precipitation must also be considered. The former reports that the growth of C_4 plants in Central Asia is associated with the arid climate[33,34]. However, most of the sites in this study, especially those above 4000 m, have annual precipitation of more than 300 mm. Obviously, it is probably not the aridity that modifies altitude trends, with C_4 plants reaching a higher altitude and low temperature sites in Qinghai-Tibetan Plateau. Perhaps the wet summer in Qinghai-Tibetan Plateau provides favorable conditions for C_4 plant. It is well known that C_4 plants have higher water use efficiency than C_3 plants in water-stress environments, and greater C_4 plant abundance occurs when low P_{CO_2} coincided with increased aridity during the last glacial maximum in America[31]. However, aridity alone is insufficient to trigger the expansion of C_4 plants in the absence of favorable climate conditions[30]. In addition, the relative abundance of C_4 plants increase by about 45% from last glacial maximum to Holocene optimum and this also proves that aridity plays the secondary role[30].

In fact, many investigations have already shown that seasonality of precipitation plays an important role rather than aridity[25—28]. Where precipitation is concentrated in the warm season, C_4 species are common, but where precipitation is concentrated in the winter and summer is dry, C_4 plants are uncommon[25—27]. The seasonal precipitation in south Tibet is obvious. The precipitation is mainly concentrated in summer. The statistic analysis of precipitation from 38 weather stations in Tibet shows that 78.9% to nearly 95% of annual precipitation is concentrated in growing season over the last 10 a. This provides not only sufficient water but also sufficient space and resource for C_4 plants, since the dry spring can often prevent C_3 species from growing.

Preliminary investigation of C_4 distribution in Qinghai-Tibetan Plateau shows that C_4 plants are present at high altitude where the minimum growing season temperature is lower than that of plain. At low P_{CO_2}, the high light and precipitation concentrating in summer favor C_4 growth at high altitude. Furthermore, it should be noted that C_4 plants perhaps exist in other place where C_4 plants are not found in this research. Therefore, further work is necessary to get a better understanding for the relationship of C_4 plants physiological character, biomass and climates.

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