Variations in leaf characteristics of three species of angiosperms with changing of altitude in Qilian Mountains and their inland high-altitude pattern

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In this study, 39 leaf samples of three angiosperms (Betula albo-sinensis, tree species; and Caragana jubata and Berberis diaphana, shrub species) were collected in the middle-east parts at 2300–3640 m asl of the Qilian Mountains to study the variations of leaf characteristics of angiosperms with altitude change in inland high-altitude regions of China. Five leaf indexes, viz. epidermal cell density (ED), stomatal density (SD), stomatal index (SI), leaf vein density (VD) and carbon isotopic ratio ($\delta^{13}$C), were analyzed in laboratory. The results show that there are significant or even very significant linear correlations between the five indexes and altitude, of which SD, SI and VD exhibit a negative correlation with altitude, while ED and $\delta^{13}$C exhibit a positive correlation with altitude. Such a correlation assemblage is quite different from the situation in the low-altitude humid environment. Generally, only an assemblage of positive correlations can be observed between the indexes (viz. SD, SI and $\delta^{13}$C, etc.) and the altitude in the low-altitude humid environment, which were caused mainly by the plants’ responses to the change of atmospheric CO$_2$ concentration ($C_a$). However, an assemblage of the negative and positive correlations found here may be attributed mainly to the plants’ responses to the change of physiological drought caused by change of low temperature, and here it is preliminarily called the inland high-altitude pattern of plant leaf variations.

Qilian Mountains, angiosperm, variations of leaf characteristics (SD, SI, ED, VD, $\delta^{13}$C), inland high-altitude pattern


Plant leaf characteristics, especially the indexes such as epidermal cell density (ED), stomatal density (SD), stomatal index (SI), leaf vein density (VD) and carbon isotopic ratio ($\delta^{13}$C) are the ideal indicators for the variations of atmospheric CO$_2$ concentration ($C_a$), air temperature ($T$) and precipitation ($P$) (Royer, 2001; Sun et al., 2003; 2009; Xie et al., 2006; Beerling et al., 2001). Related studies in the past were conducted mainly in the offshore low-altitude humid environment. Based on the classical Farquhar model (Farquhar et al., 1982) the carbon isotopic fractionation value ($J$) is closely related to stomatal flux and effective water use efficiency (WUE). Furthermore, $\delta^{13}$C with $C_a$ variation exhibits a negative correlation. On the other hand,
Woodward (1987) and Beerling et al. (1999) first found that SD and SI with $C_\alpha$ variation exhibit a negative correlation. Based on above two aspects, many scholars conducted a great deal of reconstruction work of $C_\alpha$, $T$, and $P$ since global Phanerozoic (Beerling et al., 2001; 2002; 2011; Royer, 2004; Benjamin, 2007; Sun et al., 2007; 2011). However, the relations between leaf characteristics and environmental variations that were established in offshore humid low-altitude environment by Farquhar model, Woodward and Beerling et al. are very difficult to be used in inland high-altitude region. For example, the same studies in the western part of the United States (Zuo et al., 2005) and Qilian Mountain region of China (Qiang et al., 2003; Zhang et al., 2010; Hu et al., 2010) show that the corresponding relations among $\delta^{13}C$, SD and SI with $C_\alpha$ variation are not defined. Therefore, there is an urgent need to study the relations between plant leaf characteristic indexes and altitude variation. In this study plant leaves in Qilian Mountains were selected to conduct comprehensive analysis to explore the relations between leaf characteristic indexes and altitude variations in inland high-altitude region, and to use plant fossils to provide a basis for using present things to infer the past environment in the geological period.

The study area is located in the middle-east part of the Qilian Mountains at the northeastern edge of the Qinghai-Tibetan plateau in the interior of the Eurasia (Figure 1), at the junction of East Asian monsoon region, central Asian arid zone, and the alpine frost zone of the Qinghai-Tibetan plateau. The area is quite sensitive to the climatal and environmental changes. Its north side is the Hexi Corridor and Alxa arid basin and plateau. The maximum water head of the Hei River system reaches 4000 m or more, and altitude gradient is large. The variations of environmental factors are marked with altitude gradient; therefore it is an ideal region to study how plants react to climatic environment variations (Qi et al., 2007).

Earlier related studies in the region mainly treated the gymnosperms, such as *Picea crassifolia* and *Sabina przewalskii* with a wide ecological amplitude as the objects. Qiang et al. (2003) suggested that the $\delta^{13}C$ and SD of *Picea crassifolia* below 3000 m asl are positively correlated with the altitude mainly due to the variations of $C_\alpha$, whereas above 3000 m asl they are negatively correlated with the altitude mainly due to the variations of $T$ and $P$. Zhang et al. (2010) believed that between 2600 and 3600 m asl the $\delta^{13}C$ of leaves of *Sabina przewalskii* and *Picea crassifolia* exhibits a significant positive correlation with altitude. Hu et al. (2010) thought that the $\delta^{13}C$ of leaves of *Picea crassifolia* between 2550 and 3100 m asl exhibits a negative correlation with altitude, whereas between 3100 and 3350 m asl it exhibits a positive correlation with altitude. Wei et al. (2012) put forward that SD of *Stachys sieboldii* on the south slope below 2680 m asl of the Qilian Mountains in Qinghai Province exhibits an increase variation, whereas above 2680 m asl it exhibits a decrease variation, and SI also basically exhibits a decrease variation. In one word, there is a multiplicity of views on the subject. In addition, the studies related to VD of plant leaves and leaf characteristics of angiosperms in the region are still lacking.

Angiosperm dicotyledons occupy 81% of plant species in the region, of which one tree species *Betula albo-sinensis*, and two shrub species *Berberis diaphana* and *Caragana jubata* were selected to conduct the study of leaves. *Betula albo-sinensis* is a dominant edificatory species of the region and a typical species of its characterization family of seed plants in China and world geographical flora. *Berberis diaphana* is an associated species in the forest and its family is dominant. *Betula albo-sinensis* and *Berberis diaphana* live in the relatively low forest land (2300–3200 m a.s.l.). *Caragana jubata* occurs in relatively high shrub steppe zone (3000–3800 m a.s.l.), and it is in a dominant family of the region.

### 1 Method

(i) **Field sampling.** Samples were collected in the field during July–August 2010. The latitude and longitude of the sampling area are 36°44′–38°25′N and 100°38′–103°15′E respectively, with an altitude ranging from 2300 to 3640 m asl (Figure 1 and Table 1). The sampling sites are located by hand Garmin GPS unit. According to the altitude from low to high, the bioclimatic zones of the region include semiarid mountain steppe (2300–2800 m a.s.l), mountain forest grassland (2800–3200 m a.s.l) and alpine shrub meadow (3200–3800 m a.s.l). Five trees were selected to have their sun leaves of high shoots cut at about 4 m above the ground. As for shrubs about ten plants are selected. The leaf blades are divided into two parts, one part is used for microscopic analysis and the other part is wrapped by aluminum foil to conduct $\delta^{13}C$ determination.

(ii) **Indoor sample treatment.** Includes four steps: (1) 20 perfect leaf blades were selected from each sample, cut a

\[ \text{Figure 1} \quad \text{Distribution of field sampling sites.} \quad \bullet: \text{Betula albo-sinensis; \; \star: \text{Berberis diaphana; \; \bigstar: Caragana jubata.} } \]