ISSN : 0974 - 7435 Volume 10 Issue 18

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(18), 2014 [10181-10185]

Observation on leaf epidermis micro-morphological characteristics of *Lespedeza Michx*

Bo Yuan[1]*, Ximin Cao[2]

[1]College of Science, Hebei North University, Zhangjiakou, Hebei, (CHINA)

[2]College of Agriculture and Forestry, Hebei North University, Zhangjiakou, Hebei, (CHINA)

E-mail : yuanbo197878@163.com

ABSTRACT

The epidermis of five materials from four species in Lespedeza Michx were examined and analyzed using scanning electron microscope (SEM). Three important indexes containing epidermal hair density, stomata type and stratum corneum were compared among five materials. The results show that: epidermis hairs are abundant on both upper and lower epidermis of the two species of L. cuneata and L. davurica, but only present on lower epidermis of other two species of L. cyrtobotrya and L. davidii. The shapes of five materials' leaves epidermis hairs are almost the same, there are large ecphymas with tumor appearance on all leaves epidermis hairs. The stomata of all materials besides L. cyrtobotrya sag into leaf epidermis cells, and all materials have developed stratum corneums on both sides. Drought tolerance of lespedeza closely relate to leaf epidermal hair density, sunken stomata and stratum corneum both are important reasons causing lespedeza has drought resistance ability.

KEYWORDS

Lespedeza michx; SEM; Leaf epidermis; Characteristics; Observation.

INTRODUCTION

Many leaf structural characteristics relate closely to drought tolerance, such as epidermis hair, stomata, cuticle, organization structure, vascular system and so on[1-3]. Affiliated structure of leaf epidermis, including epidermis hair and stomata, changed from leaf epidermis cells during long term development. The epidermis hair functions include reflecting unnecessary sunlight, protecting plants from sunlight illumination, enhancing protective function to epidermis, reducing moisture evaporation, maintaining and isolating moisture. Sunken stomata also plays a positive role in reducing moisture evaporation or maintaining moisture[4,5]. We know there is a close relation between epidermis characteristics and drought tolerance, therefore, we regarded it as an important index in selection of drought tolerance plant[6].

Lespedeza Michx. belongs to leguminosae perennial deciduous subshrub, shrub or herb plant. They stand 0.5-2meters high, contain approximately 100 varieties. They distribute widely in Asia, Oceania, northeast of Europe and North America[7]. There are about 70 lespedezas varieties in China, distribute in Northeast China, HeBei, NeiMeng, ShanXi, ShanXi and HeNan[8]. *Lespedeza Michx.* has many characteristics such as drought resistance, drought tolerance and impoverishment tolerance. They have developed root system to fix soil and conserve water. It is effective to hold back water drop and prevent soil from being washed for rapid growing, luxuriance branch leaves can cover large area. *Lespedeza Michx* is a fine forage bush, for resistance fixing soil, conserving water, improving ariding semiariding soil, planting seeded pasture[9,10]. In China, study on lespedeza has begun from the 1980s, mainly focused on checking forage grass resources, forestation, usage of biochemical composition, as well as stress resistance. Selecting lespedeza seed is very important for so many differences among each seed. For the first time, we studied the characteristics of the five materials' leaf epidermis hairs using SEM, and discussed mechanism of drought tolerance and provided basis for selecting lespedeza seed.

MATERIAL AND METHODS

Material

We selected 5 materials from 4 species, they are cuneate lespedeza, short stem lespedeza, big leaf lespedeza and DaWzuli lespedeza. We also selected two cuneate lespedeza materials from different produces and one lespedeza materials from other species.

Methods

We selected some even and full seeds, immersed the seeds in water for 24 hours. On 27, May, 2012, we inserted the seeds into several pots of which the diameter is 45 centimeters, and there were 15 small caverns in each pot and 6 seeds grains in each small cavern. Only a seedling was retained. The soil breeding the seedlings was countryside soil. Earlier period management was convention.

In September 2012, the strain height was about 0.5 meter, and the leaves were mature. We added sufficient water at 9:00 am and extracted the blades of leaves in the afternoon. Four species lespedeza were 3 pinnate compound leaves. We extracted top lobular leaves of mature odd compound leaves in the center. We wiped the leaves with brush to remove the dust, and abstracted the 0.5cm×0.5cm sample in the center of the vein by scissors as the experimental material.

TABLE 1 : Origin and characteristic of *Lespedeza Michx*

Species	No.	Locality	Growth characteristic	Habitat
Lespedeza cuneata (Dum. Cours.) G. Don	A17	Mississippi State	Upright small shrub	Slopes and sandy in drought
	17	YanChi NingXia		
Lespedeza davurica(Laxm.) Schindl.	14	ChiFeng NeiMenggu	Small shrubs	Slopes
Lespedeza cyrtobotrya Miq.	L13	Georgia State	shrubs	Mountain shrub
Lespedeza davidii Franch.	29	Bo Tong county HeNan	shrubs	Slopes

We cleaned the surfaces 10 seconds by ultrasonic cleaning instrument. We fixed the materials by 2.5% glutaraldehyde one night. The next day, the materials were dehydrated by alcohol of 30%, 50%, 70%, 80%, 90% and 100% 20min respectively. We fixed upper and lower epidermis of leaves on the console respectively by double sided adhesive. After air-dried and vacuum deposition, we observed and photographed them by KYKY-2800 SEM.

RESULTS AND DISCUSSION

Epidermis hair characteristics

There are epidermis hair distributing on the upper and lower epidermis of America cuneate lespedeza (Figure 1A, Figure 1B). The epidermis hair is needle hair, has lots of warty protruding objects (Figure 1C). The upper epidermis hair is sparse (11/mm^2) short (93μm), whereas the lower epidermis hair is developed (300/mm^2) and long (723μm). Both the upper and lower epidermis of Ningxia cuneate lespedeza are covered with epidermis hair (Figure 1D, Figure 1E), and it is unified non-branched hair, looks like ironclad standing upside down and leaning forward. The epidermis hair has lots of warty

protruding objects on the surface. The density of lower epidermis hair is 10 times than upper, up to 39/mm^2. Both the upper and lower epidermis of DaWuli lespedeza cuneate lespedeza are covered with epidermis hair too, is unified non-branched hair with lots of warty protruding objects on the surface (Figure 1F, Figure 1G). The density of lower epidermis hair is 10 times than upper, up to 35/mm^2. The upper epidermis hair is about 2.5μm, 0.8μm longer than lower. The short stem lespedeza has epidermis hair only on the lower epidermis. The epidermis hair is unified non-branched hair. The average length is 4.1μm, average density is 40/mm^2 (Figure 1H). There are also lots of warty protruding objects on the surface (Figure 1I). The big leaf lespedeza has epidermis hair only on the lower epidermis. The epidermis hair is unified non-branched hair. The average length is 5.3μm, average density is 13/mm^2. There are also lots of warty protruding objects on the surface (Figure 1J). The materials show that the epidermis hair of lespedeza are all unified hair and there are lots of warty protruding objects on the surface. There are differences among the epidermis hair density of different lespedeza types or lespedeza seed sources. The sequence of epidermis hair, average density being sorted from huger to litter, are America cuneate lespedeza, short stem lespedeza, Ningxia cuneate lespedeza, DaWuli lespedeza and big leaf lespedeza.

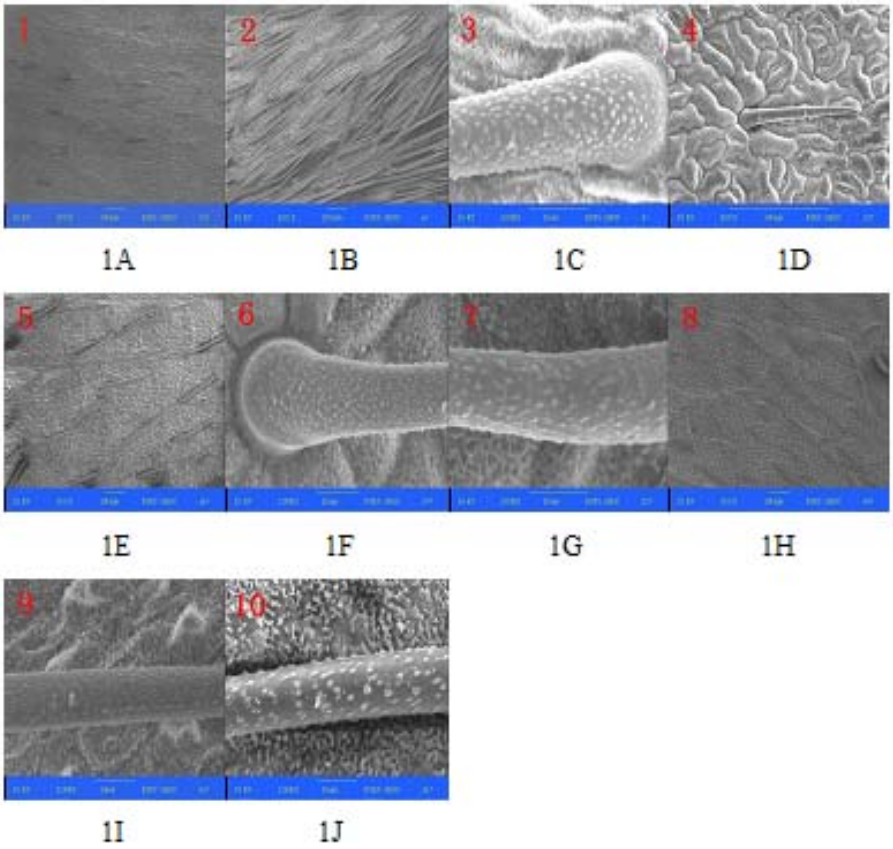

Figure 1 : Epidermis hair characteristics

Stomata characteristics

The upper epidermis stomata length of America cuneate lespedeza is 13.3μm, width is 8.3μm, density is 636/mm^2. The stomata are sunken and oval (Figure 2A). Almost the entire lower epidermis is covered with the lower epidermis hair, therefore, stomata are difficult to be found. There are stomata distributed over both the upper and lower epidermises of Ningxia cuneate lespedeza. The upper epidermis stomata length is 9.5μm, width is 3.7μm, density is 454/mm^2. The stomata are sunken and oval (Figure 2B). The lower epidermis stomata length is 10.5μm, width is 3.0μm, density is 182/mm^2. The stomata are sunken and oval (Figure 2C). There are stomata distributing over both the upper and lower epidermises of DaWuli lespedeza. The stomata are sunken and oval. The stomata of upper epidermis are bit bigger than lower. The upper epidermis stomata length is 12.0μm, width is 3.3μm, density is 364/mm^2 (Figure 2D, Figure 2E). The lower epidermis stomata length is 7.1μm, width is 2.3μm, density is 272/mm^2 (Figure 2F). There aren't stomata distributing over both the upper or lower on epidermises of short stem lespedeza, probably because the stomata are too small to detect.

There are stomata distributing over both the upper and lower epidermises of the big leaf lespedeza. The stomata are sunken and oval. The lower epidermis stomata density is 5 times than upper, and the lower epidermis stomata are bigger than upper. The upper epidermis stomata length is 15.0μm, width is 3.8μm, density is 100/mm^2 (Figure 2G). The lower epidermis stomata length is 2.3μm, width is 0.5μm, density is 22/mm^2 (Figure 2H). The common characteristics of lespedeza stomata are oval and sunken. But the size and density is not same among different seed sources.

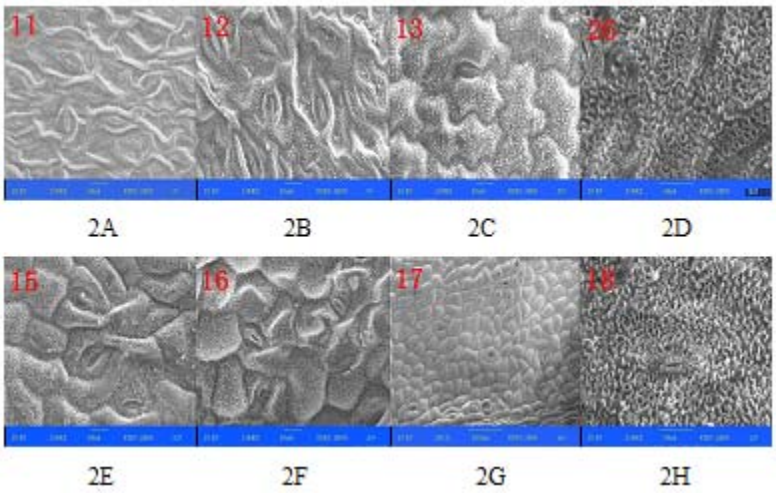

Figure 2 : Stomata characteristics

Stratum corneum characteristics

The upper epidermis of America cuneate lespedeza is covered with developed stratum corneum,which is unsmooth (Figure 3A). The lower epidermis corneum can not be observed for the lower epidermis is covered by high density of epidermal hair. The upper and lower epidermis cells of Ningxia cuneate lespedeza are all ellipse or polygonal. The surface of it is gentle andhas netted stratum corneum on it (Figure 3B, Figure 3C). The upper and lower epidermis cells of DaWuli lespedeza is wavy. The surface of it is gentle, and the upper epidermis stratum corneum looks like stripes and formed a network (Figure 3D), while the lower epidermis stratum corneumis is irregularly honeycombed (Figure 3E). The upper epidermis of short stem lespedeza is gentle while lower epidermis has netted stratum corneum (Figure 3F). The upper epidermis cells of big leaf lespedeza are regular and upper and lower epidermises both have a stratum corneum.

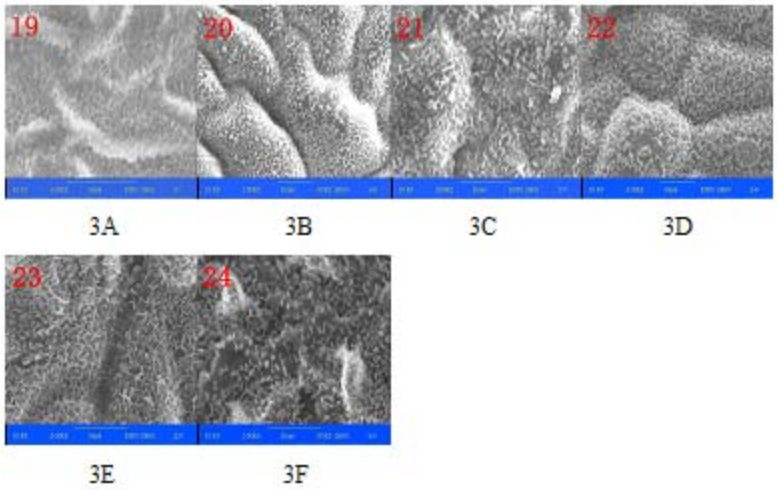

Figure 3 : Stratum corneum characteristics

Discussion

There is usuarally thick epidermal hair on leaves of xerophytes for reducing moisture evaporation and reflect intense sunlight. We found there is epidermal hair on both upper and lower epidermises on America cuneate lespedeza, Ningxia cuneate lespedeza and DaWuli lespedeza. The epidermal hair only distributed over the lower epidermis of the leaves of short stem lespedeza and big leaf lespedeza. America cuneate lespedeza has the highest density of leaf epidermal hair.

There are stomata distribute over both upper and lower epidermis of America cuneate lespedeza, Ningxia cuneate lespedeza, DaWuli lespedeza and big leaf lespedeza. We can't observed the stomata of short stem lespedeza probably because of the stomata is so small or the sunken degree of the stomata or other reason. Concerning the stomata of the materials, their common characteristics are oval and sunken. The sunken stomata is advantageous to detaining of CO_2 and H_2O, that can provide sufficient raw material for photosynthesis in xeric environment. Simultaneously, sunken stomata can prevent the moisture from outflow. It is maybe the plant nature mechanism of resisting transpiration. The characteristic is one of the important reasons why the lespedeza can possesses drought tolerance.

The water permeability of stratum corneum is so weak. In our study, stratum corneum is found in 5 materials all. It is one of the important reasons why lespedeza has drought resistance ability.

Results of SEM show, the common characteristic among 5 lespedeza materials is that, the xerophytism structure has formed on blades, a lot of epidermal hair distributing over the leaf epidermal, there is netted or honeycombed stratum corneum on epidermis cells, stomata hollow into epidermis cell.

CONCLUSION

(1)Drought tolerance of lespedeza closely relate to leaf epidermal hair density.

(2)Sunken stomata and stratum corneum both are important reasons causing lespedeza has drought resistance ability.

ACKNOWLEDGEMENT

The research projects has been subsidized by Chinese high technology research and development plan (863 plan) and Dr. Foundation of Hebei Normal University of Science and Technology and Ministry of Education emphasis laboratory Open Foundation on forest and flower genetic and breeding of Beijing forest university.

REFERENCES

[1] Song Yuxia, Yu Weiping; An anatomical study on xeromorphic structure of thedifferent life form plants in helan mountain. Acta Botanica Boreali-Occidentaila Sinica, **17(5)**, 61-68, **(2010)**.

[2] Liao Sheng Xi, Liu Juan, He Ju; A Study on the Relationship between Anatomical Structure of Leaves and Resistance Drought of Neem. Forest Research, **14(4)**, 435-440, **(2011)**.

[3] Mei Xiuying, Jiang Zaimin, Cui Hong'an; A Study on the Anatomical Structures of the Leaves of 14 Juglans regia and Juglans sigillate Varieties (Excellent Clones) and Their Drought Resistances. Journal of Northwest Forestry University, **13(1)**, 16-20 **(2011)**.

[4] Wang Liying; *Acta Botanica Boreali-Occidentaila Sinica.*, **17(5)**, 61-68 **(2012)**.

[5] Zhang Xiaoran, Wu Hong, Hu Zhengha; Relationship between morphology and structure of leaves of the main sand-fixed plants in mao us desert and environment. Acta Botanica Boreali-Occidentaila Sinica, **17(5)**, 54-60 **(2008)**.

[6] Xu Xingyou, Guo Xuemin, Du Jinyou; Observation of leaf epidermis of 3 species flowering shrubs by SEM. Journal of Hebei Science and Technology Teachers College, **19(4)**, 21-23 **(2005)**.

[7] Zhang Huiru, Wang Lijuan, Zheng Rui; Studies on relationship between five species of herbage with drought resistance and amount of proline's accumulation. Journal of Agricultural Sciences, **22(4)**, 12-14 **(2010)**.

[8] Du Jin-You, Hu Dongnan, Li Wei; Change of osmolytes in Lespedeza under drought stress. Journal of Fujian College of Forestry, **26(4)**, 349-352 **(2006)**.

[9] Wei Zhijun, Fen Guodong, Yang Jing; Observational analysis of several dry legumes pasture production performance. Journal of neimongol prataculture, **33**, 38-40 **(1994)**.

[10] Yang Zhirong, Wang Minggang, Wang Bingwen; Experimental Research on Shrub Runoff plot. Research of Soil and Water Conservation, **18(3)**, 28-30 **(2009)**.