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Leaf Anatomical Acclimation of Six Tree Species to Low Soil Water Content

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Abstract: Whether plant adaptation to soil water stress is related to their anatomical change is important to ecophysiological research. The leaf anatomical changes of six coexisting tree species (*Fraxinus mandshurica* Rupr., *Juglans mandshurica* Maxim., *Phellodendron amurense* Rupr., *Tilia amurensis* Rupr., *Ulmus japonica* (Rehd) Sarg. and *Quercus mongolica* Fisch.) from a typical community of the mixed coniferous and broadleaved Korean pine forest in Northeast China acclimated to Relative Soil Water Content (RSWC) of 100, 50 and 30% was studied. Results indicate a decrease in stomatal density for *F. mandshurica*, *J. mandshurica* and *T. amurensis* under RSWC of 30%, while the stomatal density of *P. amurense* increased. The cuticle thickness of *F. mandshurica*, *Q. mongolica*, *P. amurense* and *J. mandshurica* increased significantly under RSWC of 30%; however, a decrease in cuticle thickness was found for *T. amurensis* and *U. japonica* under RSWC of 50%. At RSWC of 30%, a decrease in the diameter of the main vein was found in *F. mandshurica* and *J. mandshurica* while an increase was seen in *P. amurense* and *Q. mongolica*. Under low RSWC, the average length of epidermal hair for *Q. mongolica*, *J. mandshurica* and *U. japonica* increased significantly, but it decreased significantly for *T. amurensis* and *P. amurense*. The palisade layer of leaves of *F. mandshurica*, *P. amurense*, *T. amurensis* increased significantly under low RSWC. The thickness of spongy layer of leaves of *F. mandshurica* and *J. mandshurica* increased significantly under low RSWC, but the thickness of spongy layer of *Q. mongolica* and *P. amurense* decreased significantly. The thickness of upper and lower epidermis did not change significantly for all tree species studied under low RSWC. These anatomical changes may have relation with leaf transpiration, but depend on species. Species anatomical divergence under drought may relate to their functional divergence.

Key words: Cuticle, epidermal hair, main vein, palisade layer, stomatal density, transpiration

INTRODUCTION

Plants lose over 90% of water taken up in transpiration. The effect of water stress on plants is the subject of intensive research because of its agronomic and ecological implications. Stress physiology research is important for improving water resource allocation^[1] and determining species drought tolerance and relative habitat adaptations^[2-5]. Under drought conditions plants generally reduce water loss by triggering stomatal closure^[6]. Based on two well established aspects of the hydraulics of stomatal function: (i) an increase in transpiration rate causes a decrease in epidermal turgor^[7,8] and (ii) a decrease in epidermal turgor causes the stomatal pore to open because of reduced “backpressure” on the guard cells^[9,10], stomatal behavior is controlled by a complex

set of anatomical and physiological determinants. Potential changes in leaf surface morphology can adapt physiological processes under drought conditions.

Fahrmy^[11] studied leaf anatomy of 20 desert plants and its relation to ecophysiology and found leaf anatomic differences (such as the ratio of palisade cell surface area/leaf or leaflet area and leaf specific conductance) between plants in different habitats. Pines derived from comparatively drier habitats in the Rocky Mountains exhibited significantly lower stomatal densities than those derived from more mesic environments in the Sierra Nevada^[12]. Schoettle and Rochelle^[13] found the significant decrease in stomatal density of *Pinus flexilis* (Pinaceae) with increasing water loss at high elevation. The difference between the ratio of intercellular air spaces to surface area in the mesophyll of plants grown under water

stress conditions has been shown to differentially influence photosynthetic rates while maintaining low values of stomatal conductance^[14].

The above research mainly focused on the investigation of plants from different environments rather than leaf anatomical variation of tree species from a same forest community. Leaves can indicate plant sensitivity to soil water gradients in an easily measurable manner. Ground observations in the area of study showed that the air temperature has increased 1.2°C in the past 50 years^[15], but precipitation has not increased. Furthermore, the climate models (general circulation models) indicate that the air temperature in Northeast China will increase and precipitation will decrease in the future because of the global climatic change^[16]. Additionally, drought is inevitable in the area because the water assumption of industrial and human activities is increasing dramatically. We predict that co-occurring tree species of the same forest community will change their leaf anatomic characteristics to acclimate the drought environment. Change would include decreased stomatal density and main vein diameter, increased thickness of cuticle, epidermal hair, palisade layer and epidermis. Meinzer^[17] suggested that functional convergence in plant response to the environment may relate with anatomical change. Therefore, we set out to determine whether a similar pattern of anatomical convergence could be detected under drought for seedlings of major co-occurring tree species (*Fraxinus mandshurica* Rupr., *Juglans mandshurica* Maxim., *Phellodendron amurense* Rupr., *Tilia amurensis* Rupr., *Ulmus japonica* (Rehd) Sarg. and *Quercus mongolica* Fisch.) in the typical mixed coniferous and broadleaved Korean pine forest dominated at this area. The aims of this research were to investigate the change in leaf micro-morphology of tree seedlings of several main tree species after short time under different soil drought conditions to see whether or not they have similar anatomical convergence; to understand the possible causes and processes of leaf anatomical acclimation to different soil water contents and if possible to determine the threshold of soil water content for leaf anatomic change.

MATERIALS AND METHODS

Seedlings: Seedlings of *F. mandshurica*, *J. mandshurica*, *P. amurense*, *T. amurensis*, *U. japonica* and *Q. mongolica* of 2 years old were transplanted from the nurseries to the greenhouse of Institute of Botany, Chinese Academy of Sciences, Beijing in May, 2000. Each plot contained one seedling and the pot size was about 20 cm of diameter and 15 cm of height. All the leaves of seedlings were cut

at the beginning of the experiment. These seedlings were grown in a moist condition for several weeks. When seedlings had 1-2 leaves the relative soil water content began to decrease. There were 10 seedlings for each treatment and total 160 seedlings in the experiment. The experiment was conducted at a semi-open greenhouse (free air and 50% sun-light could enter, but rain could not enter) and the air temperature and radiation regime changed simultaneously with the local climate. During the experimental period the Photosynthetic Photon Flux Density (PPFD) at noon was about 300-1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and the air temperature was 15- 40°C. The experiment lasted for one growing season and terminated on Oct. 8, 2000.

Water treatment: The Relative Soil Water Content (RSWC) of each pot was controlled by weighing. There were about 100, 50 and 30% treatments. Each pot (including seedling, soil and pot) had its prescriptive weight by its treatment. And each pot was weighed daily and watered to its prescriptive weight if it was below of that. Since the seedlings were relatively small and the individual weight was very limited if compared with the weight of soil and water, the effect of plant growth on adding water was not considered.

Transpiration rate: Transpiration rate of each species at each RSWC was measured on two consecutive days between 10 am and 12 pm under similar climate conditions. Ten leaves were chosen randomly and measured simultaneously (Aug. 1 and 2) using a LCi Portable Photosynthesis System (ADC BioScientific CO., UK). The average of transpiration rates of these ten leaves on the two days represented the transpiration rate of each species under each RSWC.

Leaf anatomical study: The preparations for leaf anatomical and stomatal measurement were made according to Dilcher^[18]. A brief description is given out here. Five top leaves of each treatment for each species were taken at the end of the experiment (October 2000) and fixed immediately at FFA for 24 h. The tissues were then dehydrated by an alcohol series and finally embedded in Spur's resin. Horizontal sections and cross sections near the center of the leaves were made for each sample mounted on slides and stained with Alcian and sealed. All sections were examined by light microscopy. The stomata density on lower leaf surfaces was examined in ten random view fields at a magnification of 250. The ruler in the view field was used to measure the thickness of the cuticle, palisade layer, spongy layer, upper and lower epidermis and diameter of the main vein.

Data analysis: Five replicate seedlings under each treatment were used in the experiment and all variables (such as stomatal density and cuticle thickness) were determined on 10 random view fields in each replicate. Analysis of variance (ANOVA) of SAS was used for statistical analysis. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Stomatal density: At RSWC of 30% the stomatal density of lower surface of leaves decreased significantly for *F. mandshurica*, *J. mandshurica* and *T. amurensis* (Table 1), while the stomatal density increased for *P. amurensis*. Stomatal density of *Q. mongolica* increased at RSWC of 50%, but it decreased at RSWC of 30%. For *J. mandshurica* the stomatal density at RSWC of 50% were significantly higher than it at RSWC of 30%, but for *F. mandshurica* the stomatal density of plants at RSWC of 50% was not significantly higher than those at RSWC

of 30%. The decreased stomatal density is considered an adaptation to the decreasing RSWC as it reduces transpiration.

Cuticular thickness: The cuticular thickness of *F. mandshurica*, *Q. mongolica*, *P. amurensis* and *J. mandshurica* increased significantly under RSWC of 30%, but not significantly under RSWC of 50% (Table 2). However, the cuticle thickness of *T. amurensis* and *U. japonica* decreased significantly under RSWC of 50%.

Diameter of main vein: Diameter of main vein (DMV) of *F. mandshurica* and *J. mandshurica* decreased significantly at RSWC of 30% (Table 2), but it decreased significantly for *P. amurensis* and *Q. mongolica* at RSWC of 50% and then increased dramatically at RSWC of 30%. DMV of *T. amurensis* decreased significantly under RSWC of 50% and did not change significantly for *U. japonica*.

Table 1: Leaf stomatal density and transpiration rate of different species under different Relative Soil Water Contents (RSWC)

Tree species	Species at different RSWC	Stomatal density (mm^{-2})	Transpiration rate ($\text{mmol m}^{-2}\text{s}^{-1}$)
<i>Fraxinus mandshurica</i>	Fm100	45.9 (13.29) ^A	0.544 (0.31) ^A
	Fm50	32.0 (6.86) ^B	0.705 (0.322) ^A
	Fm30	27.6 (6.14) ^B	0.905 (0.150) ^B
<i>Quercus mongolica</i>	Qm100	83.3 (12.05) ^A	0.483 (0.304) ^A
	Qm50	165.4 (19.96) ^B	0.466 (0.327) ^A
	Qm30	63.2 (12.72) ^C	0.494 (0.255) ^A
<i>Juglans mandshurica</i>	Jm100	38.4 (7.33) ^A	0.533 (0.18) ^A
	Jm50	32.8 (6.23) ^A	0.60 (0.269) ^A
	Jm30	19.7 (4.19) ^B	0.289 (0.117) ^B
<i>Phellodendron amurense</i>	Pa100	16.0 (1.69) ^A	0.609 (0.253) ^A
	Pa50	24.0 (3.14) ^B	0.728 (0.307) ^A
	Pa30	41.6 (4.08) ^C	0.711 (0.242) ^A
<i>Tilia amurensis</i>	Ta100	27.5 (3.02) ^A	0.611 (0.171) ^A
	Ta50	15.3 (4.34) ^B	0.555 (0.129) ^A
<i>Ulmus japonica</i>	Uj100	40.5 (6.06) ^A	0.722 (0.216) ^A
	Uj50	42.6 (7.98) ^A	0.688 (0.284) ^A

Values with different letters (A, B, C) at upright are significantly different ($p < 0.05$) and ones with same letter are not significantly different. Values in () are stand deviations. Sample number was 400 for stomatal density. 100: RSWC of 100%, 50: RSWC of 50%, 30: RSWC of 30%

Table 2: The thickness of cuticle, epidermal hair and main vein of different species under different Relative Soil Water Contents (RSWC)

Tree species	Species at different RSWC	Cuticle thickness (μm)	Epidermal hair length (μm)	Diameter of main vein (μm)
<i>Fraxinus mandshurica</i>	Fm100	2.72 (0.68) ^A	4.76 (0.92) ^A	856.8 (13.2) ^A
	Fm50	2.72 (0.67) ^A	13.6 (1.26) ^B	870.4 (10.2) ^A
	Fm30	6.80 (1.36) ^B	6.80 (1.45) ^A	748.0 (9.3) ^B
<i>Quercus mongolica</i>	Qm100	2.72 (0.28) ^A	1224.0 (210.3) ^A	843.2 (36.4) ^A
	Qm50	2.72 (0.24) ^A	1904.0 (312.1) ^B	571.2 (34.2) ^B
	Qm30	3.40 (0.38) ^B	2040.0 (145.7) ^B	938.4 (48.1) ^C
<i>Juglans mandshurica</i>	Jm100	2.72 (0.35) ^A	272.0 (23.9) ^A	1128.8 (110.3) ^A
	Jm50	2.72 (0.47) ^A	734.4 (53.2) ^B	1169.6 (93.2) ^A
	Jm30	3.40 (0.20) ^B	816.0 (32.8) ^B	734.4 (45.2) ^B
<i>Phellodendron amurense</i>	Pa100	2.72 (0.36) ^A	448.8 (34.6) ^A	163.2 (12.5) ^A
	Pa50	3.40 (0.66) ^A	272.0 (18.9) ^B	353.6 (23.2) ^B
	Pa30	4.53 (0.20) ^B	285.6 (15.4) ^B	1224.0 (56.3) ^C
<i>Tilia amurensis</i>	Ta100	6.80 (1.23) ^A	1768.0 (102.1) ^A	2067.2 (102.3) ^A
	Ta50	3.40 (0.54) ^B	1251.2 (95.8) ^B	788.8 (65.8) ^B
<i>Ulmus japonica</i>	Uj100	13.6 (1.32) ^A	149.6 (56.9) ^A	244.8 (22.3) ^A
	Uj50	10.88 (0.34) ^B	340.0 (76.5) ^B	272.0 (10.9) ^A

Values with different letters (A, B, C) at upright are significantly different ($p < 0.05$) and ones with same letter are not significantly different. Values in () are stand deviations. Sample number was 400 for each treatment. 100: RSWC of 100%, 50: RSWC of 50%, 30: RSWC of 30%

Table 3: The thickness of palisade layer, spongy layer, up and low epidermis of different species under different Relative Soil Water Contents (RSWC)

Tree species	Species at different RSWC	Palisade layer(μm)	Spongy layer (μm)	Up epidermis (μm)	Low epidermis(μm)
<i>Fraxinus mandshurica</i>	Fm100	13.6 (0.56) ^A	13.6 (3.5) ^A	40.8 (9.5) ^A	27.2 (6.3) ^A
	Fm50	81.6 (10.3) ^B	27.2 (6.7) ^B	27.2 (12.3) ^A	27.2 (4.6) ^A
	Fm30	108.8 (7.8) ^C	176.8 (34.8) ^C	54.4 (7.8) ^A	40.8 (8.9) ^A
<i>Quercus mongolica</i>	Qm100	95.2 (8.7) ^A	149.6 (13.4) ^A	47.6 (5.4) ^A	20.4 (3.6) ^A
	Qm50	68.0 (9.3) ^A	95.2 (10.1) ^B	34.0 (8.8) ^A	13.6 (3.2) ^A
	Qm30	81.6 (7.4) ^A	74.8 (6.5) ^C	54.4 (4.4) ^A	20.4 (4.2) ^A
<i>Juglans mandshurica</i>	Jm100	68.0 (7.6) ^A	27.2 (3.2) ^A	27.2 (4.5) ^A	27.2 (3.4) ^A
	Jm50	54.4 (4.2) ^A	68.0 (4.5) ^B	20.4 (7.1) ^A	20.4 (3.1) ^A
	Jm30	54.4 (5.2) ^A	108.8 (9.3) ^C	27.2 (3.2) ^A	27.2 (2.3) ^A
<i>Phellodendron amurense</i>	Pa100	68.0 (7.1) ^A	204.0 (17.9) ^A	27.2 (9.2) ^A	13.6 (9.1) ^A
	Pa50	136.0 (9.1) ^B	27.2 (10.1) ^B	40.8 (5.1) ^A	40.8 (10.2) ^A
	Pa30	136.0 (11.2) ^B	27.2 (4.7) ^B	40.8 (6.7) ^A	40.8 (12.1) ^A
<i>Tilia amurensis</i>	Ta100	149.6 (11.3) ^A	40.8 (6.2) ^A	20.4 (3.2) ^A	27.2 (3.9) ^A
	Ta50	190.4 (13.8) ^B	54.4 (7.3) ^A	34.0 (5.5) ^A	20.4 (4.7) ^A
<i>Ulmus japonica</i>	Uj100	136.0 (11.9) ^A	40.8 (8.9) ^A	27.2 (4.5) ^A	13.6 (7.2) ^A
	Uj50	108.8 (12.9) ^A	58.0 (9.6) ^A	27.2 (7.6) ^A	27.2 (8.6) ^A

Values with different letters at upright (A, B, C) are significantly different ($p < 0.05$) and ones with same letter are not significantly different. Values in () are standard deviations. Sample No. = 400 for each treatment. 100: RSWC of 100%, 50: RSWC of 50%, 30: RSWC of 30%

Epidermal hair: Average Length of Epidermal Hair (ALEH) of *Q. mongolica*, *J. mandshurica* and *U. japonica* increased significantly under decreasing RSWC (Table 2), while the ALEH of *T. amurensis* and *P. amurense* decreased significantly with decreasing RSWC.

Palisade layer: The palisade layer of leaves of *F. mandshurica*, *P. amurense* and *T. amurensis* increased significantly under decreasing RSWC of 30% (Table 3). The palisade layer of *J. mandshurica*, *Q. mongolica* and *U. japonica* showed no significant decrease under low RSWC.

Spongy layer: The thickness of the spongy layer of leaves for *F. mandshurica* and *J. mandshurica* increased significantly under decreasing RSWC (Table 3). While the thickness of spongy layer for *Q. mongolica* and *P. amurense* showed a significant decrease at RSWC of 50%. The thickness of spongy layer for *U. japonica* and *T. amurensis* did not change significantly.

Upper and lower epidermis: The thickness of upper and lower epidermis did not change significantly for all species under decreasing RSWC (Table 3). This indicates that the thickness of upper and lower epidermis has no relationship to RSWC or is not sensitive to decreasing RSWC.

Transpiration rate: Different tree species displayed quite differential transpiration behaviors to low RSWC. For *J. mandshurica* the transpiration rate decreased significantly under RSWC of 30% ($p < 0.01$) (Table 1), but for *F. mandshurica* the transpiration rate increased significantly ($p < 0.05$). For other species the transpiration rate did not change significantly but increased slightly for *P. amurense* and *Q. mongolica* and decreased slightly for *T. amurensis* and *U. japonica*.

DISCUSSION

Stomatal density change under decreasing soil water content:

Leaf stomatal density is an important ecophysiological parameter that affects gas exchange and transpiration. Stomata arise through differential divisions in the protoderm which becomes secondarily meristematic forming guard mother cell. The governing mechanisms are not fully understood, but the placement of division planes in parent cells leads to the formation of stomata^[19]. Giles and Shehata^[20] found that cell division and elongation in *Zea mays* were related to the plastochron index and that guard mother cell differentiation was linked to the potassium pump. Friend and Woodward^[21] hypothesized that stomatal density responses were under the mechanistic control of a substance such as ATP. Poole *et al.*^[22] indicated that air humidity might affect the development of stomata. In this research, after acclimation to low RSWC for about one growing season, the stomatal densities of tree species responded differently. Generally, under decreasing RSWC stomatal conductance and transpiration decreased. Körner *et al.*^[23,24] found that herb, shrub and broad-leaf tree species in wet conditions had increased stomatal density and different ratios of ¹³C to ¹²C compared to those at dry conditions. In this study for *F. mandshurica*, *J. mandshurica* and *T. amurensis* the stomatal density of leaves decreased significantly under decreasing RSWC; however, for *P. amurense* the leaf stomatal density increased under decreasing RSWC. *Q. mongolica* increased its stomatal density at RSWC of 50%, but it decreased at RSWC of 30%. The stomata density of *U. japonica* changed not significantly under decreasing RSWC. The stomatal density change might be related to the sensitivity of decreasing RSWC for tree species. Mitton *et al.*^[25] found that the variation in allozymes and stomatal size in pinyon (*Pinus edulis*, Pinaceae) associated with soil moisture.

Two explanations were proposed, one focused on metabolites and the other focused on stomata. Cobb *et al.*^[26] pointed out that plants produce serine, one of the precursors of glycinebetaine, which is accumulated in response to drought stress. Different genotypes in pools of glycinebetaine (Gly) may produce differences in cell turgor and the capability to maintain normal physiological processes under drought stress. The second explanation was the variation in stomatal size and shape among *Gly* genotypes. The relationship between stomata density and stomatal conductance needs further research.

Change of cuticle, epidermal hair and main vein under decreasing soil water content: Plant cuticle and epidermal hair constitute the interface between plant tissue and the environment. Based on previous ecological studies, it is apparent that the leaf hair and cuticle are adaptive features of plants to arid conditions^[27,28]. The thick cuticle and epidermal hairs are useful to restrict water loss in leaves under drought conditions. In this study we found that the cuticle thickness of *F. mandshurica*, *Q. mongolica*, *P. amurense* and *J. mandshurica* increased under RSWC of 30%, but did not change significantly under RSWC of 50%. However, the cuticle thickness of *T. amurensis* and *U. japonica* decreased significantly under RSWC of 50%. ALEH of *Q. mongolica*, *J. mandshurica* and *U. japonica* increased significantly under water stress and ALEH of *F. mandshurica* did not change significantly, but ALEH of *T. amurensis* and *P. amurense* decreased significantly. Boundary layer resistance due to hairs is commonly quantified by dividing the thickness of the pubescent layer by the diffusion coefficient of water vapor in the air^[29]. Further more, decreased DMV is useful in conserving water. At RSWC of 30% DMV decreased significantly for *F. mandshurica* and *J. mandshurica*, increased significantly for *P. amurense* and did not change significantly for *Q. mongolica*. DMV of *T. amurensis* decreased significantly under RSWC of 50% and it did not change significantly for *U. japonica*. Micro-morphological adaptations to drought include variations among cuticle, DMV and epidermal hairs. Not every tissue should be individually optimized, but plants can be expected to have evolved tissue arrangements for most efficient way^[30,31].

Change of palisade layer, spongy layer and upper and lower epidermis under decreasing soil water content: The thickness of palisade layer and spongy layer was sensitive to water stress, but the thickness of upper or lower epidermis was not sensitive to decreasing RSWC. Leaf thickness can be used to detect water stress for

different tree species. For *F. mandshurica*, *P. amurense* and *T. amurensis* the palisade layer increased significantly under low RSWC, but did not change significantly for *J. mandshurica*, *Q. mongolica* and *U. japonica*. The spongy layer of *F. mandshurica* and *J. mandshurica* increased significantly under low RSWC, but decreased significantly for *Q. mongolica* and *P. amurense* and it did not change significantly for *U. japonica* and *T. amurensis*. Leaf anatomical changes are assumed adaptations to water stress, because rates of CO₂ assimilation are regulated by the intrinsic photosynthetic capacity of the mesophyll and by CO₂ transfer conductance from ambient air to carboxylation sites in chloroplasts^[32]. Chartzoulakis *et al.*^[14] found that the difference of mesophyll volume and the surface area of mesophyll cell walls could affect the CO₂ transfer and photosynthetic rate in two olive cultivars. In this study, the anatomical structure of different species from the same forest community responded differently. The different responses may partly explain the difference between species tolerance of water stress.

Leaf anatomical change and transpiration rate: In order to maintain leaf growth under stress condition, plants may possess plasticity to alter leaf anatomical properties and stomatal density^[33]. For *J. mandshurica* the relationship between leaf anatomical change and transpiration is obvious and supports our assumption well. Its stomatal density and diameter of main vein decreased under RSWC 30% and cuticle, epidermal hair, palisade layer and spongy layer increased; then, the transpiration rate would possibly decrease based on our assumption. In fact, the transpiration rate of *J. mandshurica* did decrease as RSWC decreased. For *P. amurense* the stomatal density, diameter of main vein and palisade layer increased, epidermal hair and spongy layer decreased. It was consistent with the slight increased transpiration rate. However, for *F. mandshurica* the transpiration rate increased, but the stomatal density and diameter of main vein decreased and the thickness of cuticle, epidermal hair, palisade layer and spongy layer increased. Similar with *T. amurensis*, its transpiration rate decreased slightly but its cuticle and epidermal hair decreased. Study on Velvet-grass indicated that hair and stomatal density had their maximum mean value around 50% of RSWC; hair and stomatal density were positive corrected^[34]. The different anatomical change and transpiration acclimation is a kind of anatomical and functional divergence, which may be ultimately related to species adaptation and survival. This study has shown that the correlation between leaf anatomical change and low soil water content depends on plant species and there exist diversity of drought adaptation among species^[35]. Multiple anatomical changes

should be considered as a selection criterion to assess plant drought tolerance.

In this study, it appeared that different tree species acclimated to soil drought by changing their leaf anatomical characteristics differently. For majority of tree species RSWC of 30% was close to the threshold of anatomical and transpiration change. Some species acclimated to drought condition by the expected ways, which mean to decrease stomatal density and diameter of main vein and increase thickness of cuticle, epidermal hair, palisade and spongy layer, such as *J. mandshurica*. However, some species had more complicated way to acclimate to drought condition. Their separated anatomical index change might not be fully explained in their functionality.

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