PLANTS
IN URBAN AREAS AND LANDSCAPE

THE STOMATAL RESPONSE OF *GINKGO BILOBA* L. TO WATER STRESS

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The goal of the presented study was to define stomatal closure and water content changes in the leaves of *Ginkgo biloba* L. in relation to dryness of the soil substratum. We supposed that ginkgo would preserve relative water content in the leaves by stomatal closure after detection of water scarcity in the soil substratum. Relative water content in the leaves, water content of the soil substratum and stomatal conductance have been measured. The results showed that ginkgo was able to keep high relative water content in the leaves long time after water scarcity introduction. During the first three weeks under the conditions of water scarcity there has been observed relatively high water content in the leaves; its value was about 94%. In relation to the soil desiccation *Ginkgo biloba* L. plants reacted by the decrease of stomatal conductance. After the first week without water when water capacity of the soil substratum reached 58% (from 75% at the beginning of the experiment) stomatal conductance decreased on 28% (from 0.86 mm/s^1 to 0.62 mm/s^1). The decrease in stomatal conductance has been observed in all next days. The most significant results have been observed during the first two weeks of the experiment.

Keywords: *Ginkgo biloba* L., stomatal conductance, relative water content, drought

1 Introduction

Drought is the most important environmental stress, severely impairing plant growth and development (Anjum et al., 2011). It is known that drought significantly influences photosynthesis by changes in metabolism and regulation of stomatal conductance (Bota et al., 2004).

Stomata have two main functions in the plant. They participate in photosynthesis course and optimise water balance. Stomata open after sunrise (when there is light for photosynthesis) and close after sunset (Procházka et al., 1998). Stomata play key role in retaining water in plants. Stomatal closure is the main cause of transpiration restriction in increasing water stress (Hetterington and Woodward, 2003), which results into thedecrease of leaf cooling and lower nutrients intake and transport (Arve et al., 2011).

Stomatal control of water loses was identified to be one of the first reactions of plants to water deficit which leads to the reduction of CO_2 uptake through leaves. Stomatal closure occurs at a loss of turgor with the decrease of water potential or by influence of low water content in the environment. Stomatal closure closely related to soil water content. Stomata react on chemical signals (abscisic acid – ABA) produced by dehydration of root systems while in the leaves constant water level is maintained (Chavez et al., 2002). It is assumed that ABA induces a signal in plants which helps them reduce the speed of transpiration (Lee, 2013). Except of leaves can be in conditions of water scarcity ABA synthetized in roots and through xylem is transported to leaves which subsequently dry (Slováková and Mistrík, 2007). Stomatal closure is directly linked with low water content in the soil (Chapman and Auge, 1994)

One of indicators of metabolic changes in the plants is a change in relative water content (*RWC*). *RWC* represents the total amount of water needed by the plant at full saturation. The *RWC* expresses the water content in per cent at a given time as related to the water content at full turgor (González and González-Vilar, 2001). The decrease of *RWC* is accompanied with changes in physiological functions of plants, synthesis of growth and stock substances and metabolic changes. The relationship between *RWC*, water content in the soil substratum and stomatal conductance could be an indicator of plants reactions to water stress.

One of the species, generally considered as the most adaptable to environment is *Ginkgo biloba* L. *Ginkgo biloba* L. originates from Southeast Asia. Its natural habitat is the eastern China. It grows in wide range of soils from sandy to heavy loamy losses and

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prefers sunny or semi shade sites (URL 1). PH value of substratum tolerates the range from 5.5 to 7.7. The recessive critical point of damage influenced by acid rains reaches the pH 3.0 (Hu et al., 2010). The lack of water causes changes in nutrients amount (Wang et al., 2005), growth inhibition (Zhang et al., 2005) and changes in growth and biomass accumulation in favour of the root system (Jing et al., 2005).

From the previous studies we know that stomatal conductance of *Ginkgo biloba* leaves is significantly lower in shaded leaves compared to the leaves exposed to direct sunlight. (Sarijeva et al., 2007). *Ginkgo biloba* reduces stomata under the influence of higher concentration of CO_2 (Beerling et al., 1998). Photosynthetic activity of leaves is the highest from May to July (Tao et al., 1999) and probably linked to the chlorophyll content in leaves, which is the highest at the end of shoot growth at the turn of June and July (Raček et al., 2014). It is also known that the decrease of soil water capacity on 40% causes chloroplast degradation (Wang et al., 2008). There is little available knowledge about the influence of water scarcity on stomatal closure in relation to the ability to preserve leaves from water losses. The goal of the presented study was to define stomatal closure and water content changes in the leaves in relation to dryness of the soil substratum. We supposed that ginkgo would preserve *RWC* in the leaves by stomatal closure after the detection of water scarcity in the soil substratum. The results bring new knowledge in respond to ginkgo to water stress and are applicable in the area of plant cultivation and maintenance in urban forestry systems.

2 Materials and Methods

The plant material was produced from seeds. The donor parent tree was growing in the park of Topoľčianky in the south of Slovakia. It were two-year old plants one year grown in 3l containers. TS 3 standard substratum (pH 5.5 to 6.0 + fertilizer 1 kg/m^3) enriched by clay fraction (0–25 mm / m clay 20 kg/m^3) was used. Until the end of June the humidity of soil substratum was on the level of 75% of soil water capacity. From the mid-June (at the beginning of experiments) the irrigation was stopped and the process of continual soil desiccation began. Irrigation was stopped until the end of experiments (for the next 35 days). During the experiment the plants were stored in a greenhouse, preserved from the rain and full sunlight.

Stomatal conductance, as an index of plant stress and the indicator of photosynthetic activity, was measured with the AP4 Leaf Porometer. Measurements were taken twice a week on two fully expanded leaves of five plants between 07.00 and 10.00 hours. Ten replications were made for each measurement.

Once a week there were defined the water capacity of the soil substratum and the relative water content in the leaves. There were used two plants for analyses each weak. Water contents were set gravimetrically, *RWC* was set as follows:

All leaves per plant were detached to determine their relative water content (*RWC*). After cuttings, the petiole was immediately immersed in distilled water inside of a glass tube, which was immediately sealed. The tubes were then taken to a laboratory where the increased weight of the tubes was used to determine the leaf fresh weight (*FW*). After 4h, the leaves were weighed to obtain the turgid weight (TW). The dry weight (*DW*) was then measured after oven drying at 80ºC for 48 h, and *RWC* was calculated as follows: *RWC* =100(*FW* - *DW*)/ *TW* - *DW*. After the end of the experiment, the analysis of variance and multiple range tests were used for the data evaluation. The software Statgraphics Centurion XVI was used for statistical evaluation.

3 Results and Discussion

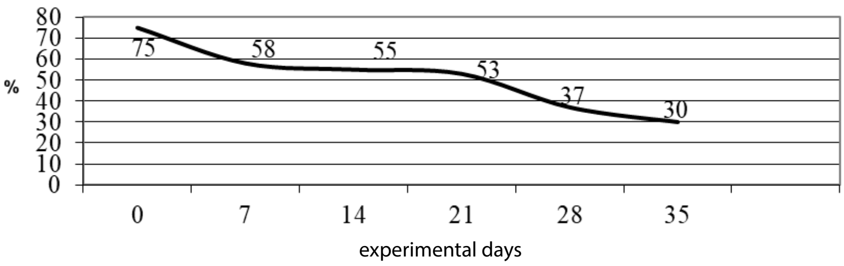
The relationships between soil substratum desiccation, stomatal conductance and *RWC* in the leaves were analysed. We found out that the stomatal conductance of *Ginkgo biloba* L. plants decreased in relation to humidity of soil substratum in accordance with our supposition. Soil water capacity was 75% at the beginning of the experiment. When irrigation was stopped after a week, soil water capacity decreased on 58%. After the second week the soil water capacity decreased on 55%. Soil desiccation continued in the next weeks. After five weeks (35 days), the soil water capacity finally decreased by 30%.

In relation to soil desiccation, *Ginkgo biloba* L. plants reacted by linear decrease of stomatal conductance. After the first week without water, stomatal conductance decreased on 28% (from 0.86 mm/s^{-1} to 0.62 mm/s^{1}) after second week for next 28% (from 0.62 mm/s^{-1} to 0.38 mm/s^{-1}). The decrease in stomatal conductance has been observed in all following days, and after five weeks (35 days), the stomatal conductance decreased by 0.08 mm/s^{-1} (Table 1). The most significant results have been observed during the first two weeks of experiment.

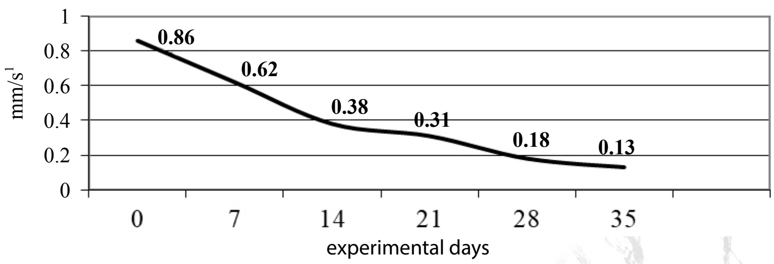
During the first three weeks of the experiment there has been observed high *RWC* in leaves; its value was about 94%. The decrease of *RWC* was observed after 21 days without irrigation when *RWC* began to change more

Table 1 Multiple range test of stomatal conductance in *Ginkgo biloba* L. leaves under water stress (P-value < 0.05) in weekly intervals

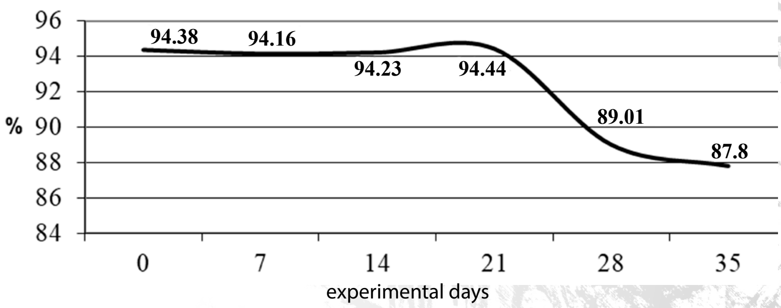
Experimental days	Count	Mean (mm/s[1])	Groups differences
0	10	0.86	*
7	10	0.62	*
14	10	0.38	*
21	10	0.31	* *
28	10	0.18	* *
35	10	0.13	* *



Decrease of water capacity in the soil substratum during dessication



Decrease of stomatal conductance of *Ginkgo biloga* L. leaves during soil substratum dessication



***RWC* development in the leaves of *Ginkgo biloba* L. during soil substratum dessication**

■ **Figure 1:** Development of water capacity in the soil substratum, stomatal conductance, *RWC* content in the leaves of *Ginkgo biloba* L. under conditions of water stress

significantly. *RWC* decreased on 87.8% at the end of the experiment.

There is a lack of knowledge about the right influence of the soil water content on the decrease of stomatal conductance of *Ginkgo biloba*. It is known that dryness and high temperatures cause the decrease of stomatal conductance in young ginkgo plants (Zhang et al., 2005); especially the decrease of field water capacity from 60% to 40% meant significant decrease of stomatal conductance against plants growing in the soil with field water capacity on 80% and 60% (Mao, 2005). Stomatal conductance decreased after the decrease of the soil water capacity under 60%. Our results show that the significant decrease of stomatal conductance was observed already by the decrease of the soil water capacity on 60%. Results show that ginkgo plants reacted effectively by stomatal closure on the decrease of the soil water content. That reaction indicates the ability to effectively manage water in plants. Subsequently, the decrease of stomatal conductance in relation to 60% soil water content when there were not observed more significant changes in *RWC* is of importance.

It is well known that the leaf water status always interacts with stomatal conductance (Anjum et al., 2011). Increased stomatal sensitivity is a functional mechanism that allows plants to maintain high water status during drought periods (Pessarakli, 2002). The rate of *RWC* in plants with high resistance against drought is higher than in others (Arjenaki et al., 2012). Based on this knowledge and our results we can define *Ginkgo biloba* L. as a species with high tolerance to drought.

Plants long term maintained high *RWC*, during the first weeks without water it was 94%. The change of *RWC* occurred after three weeks without water, when the soil water content decreased on 50% of the soil water capacity. *RWC* in the leaves was still high, approximately on 89%. *RWC* is closely related to photosynthesis limitation. A border of photosynthesis limitation in relation to *RWC* could be very variable and reactions of plants are specific due to species differences (Bota et al., 2004; Flexas et al., 2006). Chaves et al (2002) consider the decrease of *RWC* on 70–75% as a limit for photosynthesis. Due to the fact that ginkgo was able to keep high water content in the leaves it is most likely that limit for slowdown or stop of photosynthesis is a significant decrease in stomatal conductance or the restriction of transpiration and CO_2 uptake.

4 Conclusions

The results show that because of stomata closure, the examined plants had sufficient amount of water during the first weeks under water scarcity. This reaction represents an important advantage for *Ginkgo biloba* in the environment with lack of or unevenly distributed water. The retention of high *RWC* in the leaves allows waiting out periods with lack of water without destruction or with limited destruction of leaves. The significant limitation of transpiration by stomata closure limits photosynthesis and growth, but also allows ginkgo to get over periods of water scarcity.

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