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Effects of some Stress Factors (Aluminum, Cadmium and Drought) on Stomata of Roman Nettle (*Urtica pilulifera* L.)

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Abstract

In this study, Roman nettle (*Urtica pilulifera* L.) seedlings grown singly in standard pots containing compost were exposed to two different levels of aluminum and cadmium (100 µM and 200 µM) and water stress (moderate and severe stress) treatments. Measurements of stomatal perimeters, diameters and areas from the epidermal sections in lower surfaces of young expanded leaves of main stem and first lateral branches were examined by image processing and analysis software. The data proved that all stomata were affected significantly, but with varying responses, in all treated plants compared to control plants. Excluding severe water stress (WS 2), the data from first lateral branch leaves showed slight sensitivity to all stress treatments. Nevertheless, there were no statistically significant differences between stomatal measurements from main stem and first lateral branch leaves. Particularly, reduction in stomatal diameters of both main stem and first lateral branches in severe water stressed plants, reducing by 26.45% and 48.09% respectively; suggest that this could be a response of *U. pilulifera* to drier environments.

Keywords: Stomata, Al, Cd, water stress, drought, nettle, *Urtica pilulifera* L.

Introduction

Stomata are the principal means of gas exchange in vascular plants. They are small pores, found epistomatically, hypostomatically and amphistomatically on leaves that are fully/partly opened or closed under the control of a pair of kidney-shaped cells called guard cells (Fitter and Hay, 1978; Grant and Vatnick, 1998; Adedeji and Jewoola, 2008). In nature, the opening and closing of stomata involves feedback and feed-forward loops, and is affected by decreased CO_2 in the intercellular air space, too much transpiration and some environmental conditions such as water stress (Garbutt *et al.,* 1990; Jones,1992). The stomatal mechanism is also affected by the plant hormones; abscisic acid (ABA), cytokinins, auxins, and possibly gibberellic acid. ABA plays an important role in stomatal closure, seed dormancy and plant adaptation to environmental stresses (Tal and Imber, 1970; Davies, 1987). In addition, stress factors like high salinity and drought are among the most crucial factors for the growth of plants and water stress induces a rapid decline in stomatal conductance, rate of transpiration and net photosynthesis (Davies, 1987; Kozlowski 1997; Munns, 2002; Buckley, 2005). Some toxic and heavy metals affect soil pH and uptake of the nutrients from the soil, which influence plant growth and development (Matsumoto, 2000; Neil and Gregory, 2001; Nocito *et al.,* 2002; Vitorello *et al.,* 2005).

Al is the most abundant metal in the earth's crust and one of the most important components of the soil (7%), and also it is soluble as a trivalent ionic form is highly active in acid soil (< pH 5.0) and toxic to plant growth (2-3 ppm) causing reductions in crop production (Thornton *et al.,* 1986; Kochian, 1995; Matsumoto, 2000; Vardar *et al.,* 2006). The molecular mechanisms of Al toxicity are still poorly understood, despite extensive studies (Rengel, 1992; Delhaize and Ryan, 1995). Among the common effects of Al are: decrease in total leaf number and size, a decrease in shoot biomass, inhibition of root elongation, chlorosis and necrosis of leaves leading to decreased photosynthetic activity (Thornton *et al.,* 1986; Kochian, 1995; Jones and Kochian, 1995). Al also causes ultrastructural and cellular changes in leaves, as cell division and elongation are inhibited, and reduces stomatal aperture (Rengel, 1992; Kochian, 1995; Delhaize and Ryan, 1995; Vardar and Ünal, 2007).

Cadmium is considered a trace element, and is one of the heavy metals with an occurrence in natural and agricultural environments mostly resulting from human activities, such as industrial processes like mining and refining (Wagner, 1993; Sandalio *et al.,* 2001; Akgüç *et al.,* 2008). Cd is a strong phytotoxic element, which inhibits vegetative plant growth and even causes plant death (Sandalio *et al.,* 2001). The mechanisms involved in cadmium toxicity still require more research, despite intensive studies on its toxicity in a variety of plants. Common effects of Cd include; affecting water balance of plants by reducing root growth, limiting water uptake via a reduction in vessel size, and causing partial stomatal closure (Barcelo and Poschenrieder, 1990;

Prasad, 1995). It also causes a decrease in tissue biomass, chlorosis, and effects on specific physiological (e.g., xylem transport) or biochemical (e.g., nitrogen fixation) processes (Kosma *et al.,* 2004).

Water stress is also one of the most important environmental factors causing to reduction in plant growth and development as well as plant productivity and crop yields (Boyer, 1982; Jones and Famjul, 1982; Akıncı, 1997). The effect of water stress can be manifest in many ways, as varied morphological, physiological and biochemical changes in plants under different water stress. For instance changes in leaf morphology (Parker, 1968; Morgan, 1980; Hsiao *et al.,* 1984; Blum,1989; Akıncı, 1997), effects on shoot and root growth and development (Sharp and Davies, 1979; Rambal and Debussche, 1995; Akıncı, 1997), limiting photosynthetic activity by decreasing CO_2 influx, decrease in carboxylation, electron transport chain activities of the chloroplasts in the mesophyll cells (Akıncı, 1997). It also affects many other metabolic pathways, mineral uptake, membrane structure (Schulze, 1986; Davies and Zhang, 1991; Tardieu and Davies, 1993; Davies, 1995) stomatal structural changes and conductance (Huber *et al.,* 1984; Wong *et al.,* 1985; Raschke and Resemann, 1986; Cornic *et al.,* 1989; Akıncı, 1997), and CO_2 uptake (Hsiao, 1973; Quick *et al.,* 1992; Akıncı, 1997).

Water deficit in plants causes the closure of stomata (Hsiao, 1973; Epstein and Grant, 1973; Quick *et al.,* 1992; Akıncı, 1997), which decreases both transpiration and photosynthesis in many plant species (Zelitch, 1971; Shekharv and Iritani, 1979; Fatemy *et al.,* 1985). Stomatal closures occur via the distress signal "abscisic acid" and lead to a decreased rate of transpiration from the mesophyll chloroplasts to the guard cells of the stomata during water stress conditions (Wright, 1969; Wright and Hiron, 1969).

Urticaceae family members are very common and widespread species found in the margins of arable fields, gardens and countryside throughout Europe, Asia and Northern Africa (Firbank *et al.,* 2002). They have high nutrient requirements demonstrated by leaves, which contain high levels of N, Ca, Mg (Grime *et al.,* 1988; Wilman and Riley, 1993) and Fe (Salisbury, 1962). *Urtica dioica* L. (stinging nettle) and *Urtica urens* L. (dwarf nettle) are well-known *Urticaceae* family member species and they have been used as medicinal plants all over the world for years (Kavalalı *et al.*, 2003). They have used as expectorant, purgative, diuretic, hemostatic, vermifuge and for the treatment of eczema, rheumatism, hemorrhoids, hyperthyroidism, bronchitis and cancer (Barker, 2001; Kavalalı *et al.,* 2003). Furthermore, their stems have also used for making linen and ropes (Bond *et al.,* 2006). A less known *Urticaceae* family member *U. pilulifera* L. (Roman nettle) locally, named "Kara Isırgan" is one of the most important traditional drugs in Turkey. All parts of the plant bristle with stinging hairs and it flowers from May to August (Davis, 1982). In Turkish traditional folk medicine, this plant is commonly used as a remedy for diabetes mellitus (Baytop, 1999). Up to this day, such use of *U. pilulifera* L. is quite prominent in the Black Sea region of Turkey (Kavalalı *et al.,* 2003).

The objectives of this research were to investigate the effects of different levels of Al (100 μM-200 μM), Cd (100 μM-200 μM) and water stress (moderate stress and severe stress) exposure to Roman nettle seedlings and to observe the relationship between some stomatal parameters (pore diameter, perimeter and area) and various stress types under growth room conditions.

Materials and methods

Growing seeds

The surface of Roman nettle seeds were soaked by immersion in ethyl alcohol (50%) for 1 minute followed by deionized water for 5 minutes. They were then transferred into small vessels containing sterilized compost for germination. During the germination period (2 weeks), the seeds were moisturized with deionized water. When the shoot lengths of the young plantlets reached 3-4 cm, they were transferred into standard plastic pots containing sterilized compost and maintained under growth-room conditions. The plants were grown under fluorescent tubes giving an irradiance of 5000 lx (day/night-16/8 respectively), and a temperature of 23±2 °C and relative humidity 45-50%. Each of the experimental groups of eight replicates were watered with Hoagland's nutrient solution (Hoagland and Arnon, 1950) at two-day intervals for the 2 months during which the stress treatments were applied.

Stress Treatments

Application of Al and Cd

While control plants were watered only with Hoagland solutions, the experimental groups were watered with spiked Hoagland solutions (prepared as 100 and 200 μM $AlCl_3$ or $CdCl_2$). Each treatment was watered with 40 ml of solution at two-day intervals. The soil pH was adjusted to 4.5 for Al treatments using 2% H_2SO_4.

Water stress

The gravimetric determination of water content by weighing soil samples before and after oven drying to constant weight at 85 °C was used to calibrate all measurement of the moisture content of compost in pots. The pot weights corresponding to soil moisture contents after 12 and 18 days were calculated according to the equation of Paquin and Mehuys (1980). After determining the stress levels as 52% and 45% moisture content for moderate (MS) and severe stress (SS) levels respectively, the seedlings were watered at two-day intervals to maintain the moisture levels.

Tab. 1. The reduction % of treatments of main stem and first lateral branches with respect to controls (D= Diameter, P= Perimeter and A= Area)

	Al 1			Al 2		
Reduction %	D	P	A	D	P	A
Main Stem	60.31	33.15	74.42	61.86	36.96	73.84
Branch	64.39	43.09	77.75	69.55	38.79	79.84
	Cd 1			Cd 2		
Reduction %	D	P	A	D	P	A
Main Stem	43.19	21.58	55.92	60.70	38.40	73.68
Branch	56.02	18.65	57.12	68.71	24.73	71.07
	WS 1			WS 2		
Reduction %	D	P	A	D	P	A
Main Stem	70.3	34.74	76.64	26.45	29.31	58.30
Branch	68.51	55.66	81.20	48.09	36.60	61.08

After 2 months of stressing with Al, Cd and water, plants were harvested and microscopic preparations were arranged for stomata studies. The two youngest fully-expanded leaves from shoots and first lateral branches were harvested from each plant, and for each leaf 20 stomata from the abaxial leaf epidermis were measured for stomatal apertures (pore diameter, perimeter and area) (Fig. 1). The preparations were photographed with an Evolution LC Color camera and an Olympus BH-2 microscope. The images were analyzed with Image-Pro express version 6.0 scientific image processing and analysis software. The stress treated plants are abbreviated as Al 1 (100 μM $AlCl_3$), Al 2 (200 μM $AlCl_3$), Cd 1 (100 μM $CdCl_2$), Cd 2 (200 μM $CdCl_2$), WS 1 (moderate water stress) and WS 2 (severe water stress) respectively.

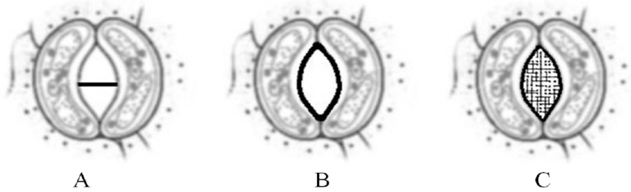

Fig. 1. Different stomatal measurements A) Pore diameter, B) Perimeter, C) Area

Statistical Analysis

The data were subjected to paired-sample T-tests, using SPSS 11.5 for Windows, with 95% ($P < 0.05$) significance of differences between means. Means are indicated with standard error (bars indicate s. e.).

Results and discussion

In this study, the diameters, perimeters and areas of stomata from the lower surfaces of leaves were measured using Image-Pro express version 6.0 scientific image processing and analysis software. In all cases, stomatal sizes were significantly decreased in stress-imposed replicates compare to control values (Fig. 2-3). The reduction in the values for first lateral branch leaves stomata for Al 1-2 and WS 1 treatments were slightly higher than the reduction in the main stem stomata for these treatments (Tab. 1). The reduction of the stomatal sizes in Al 1 and Al 2 showed similarities, indicating stability of effect, unlike Cd 1-2, and WS 1, which particularly reduced stomatal openings (Vitorello *et al.,* 2005). The changes in Al treated plants suggest the inhibition of K^+ in guard cells, which is correlated to stomatal opening (Schroeder, 1988; Schroeder *et al.*, 1994). Al treatment of plants (9 h) induced stomatal closure (Sivaguru *et al.,* 2003) and abscisic acid regulates potassium and chloride ion channels at the plasma membrane of guard cells, leading to stomatal closure by reducing transpiration (Leyman *et al.,* 1999). Comparing the values between the main stem and first lateral branch leaves, it was observed that the differences between decreasing values in all stomatal measurements fluctuated in WS 1, Cd 1 and Cd 2 (Tab. 1). The results of Cd 1 and Cd 2 treatments suggest that water absorption level was affected by Cd, as well as ABA changes, leading to stomatal closure and significant decrease in stomatal opening with increased Cd concentration. It has been suggested that Cd has a direct effect on the ion and water movement in the guard cells (Sayed, 1997); nitrogenase activity declined (30%) even at18 μM, and photosynthesis was depressed by 60% by 300 μM Pb and Cd (Huang *et al.*, 1974). It has also been reported that Cd reduces ATP and chlorophyll concentrations in many species, decreases oxygen production (Das *et al.,* 1997), and that significantly reduced transpiration rates (Sayed, 1997) might be related to Cd-treated plants having smaller stomatal apertures (Huang *et al.,* 1974; Sayed, 1997).

The various effects of water deficit seen on stomatal structure are clearly mechanisms to enable plants to survive in stress conditions. For instance, various strategies can be seen in wheat and other cereals in terms of turgor loss and stomatal closure at different relative water content (Richter and Wagner, 1982). In this study, Roman nettle seedlings responded differently to two levels of water stress. The first level of water stress (WS 1) caused the greatest reduction in main-stem leaf stomatal param-

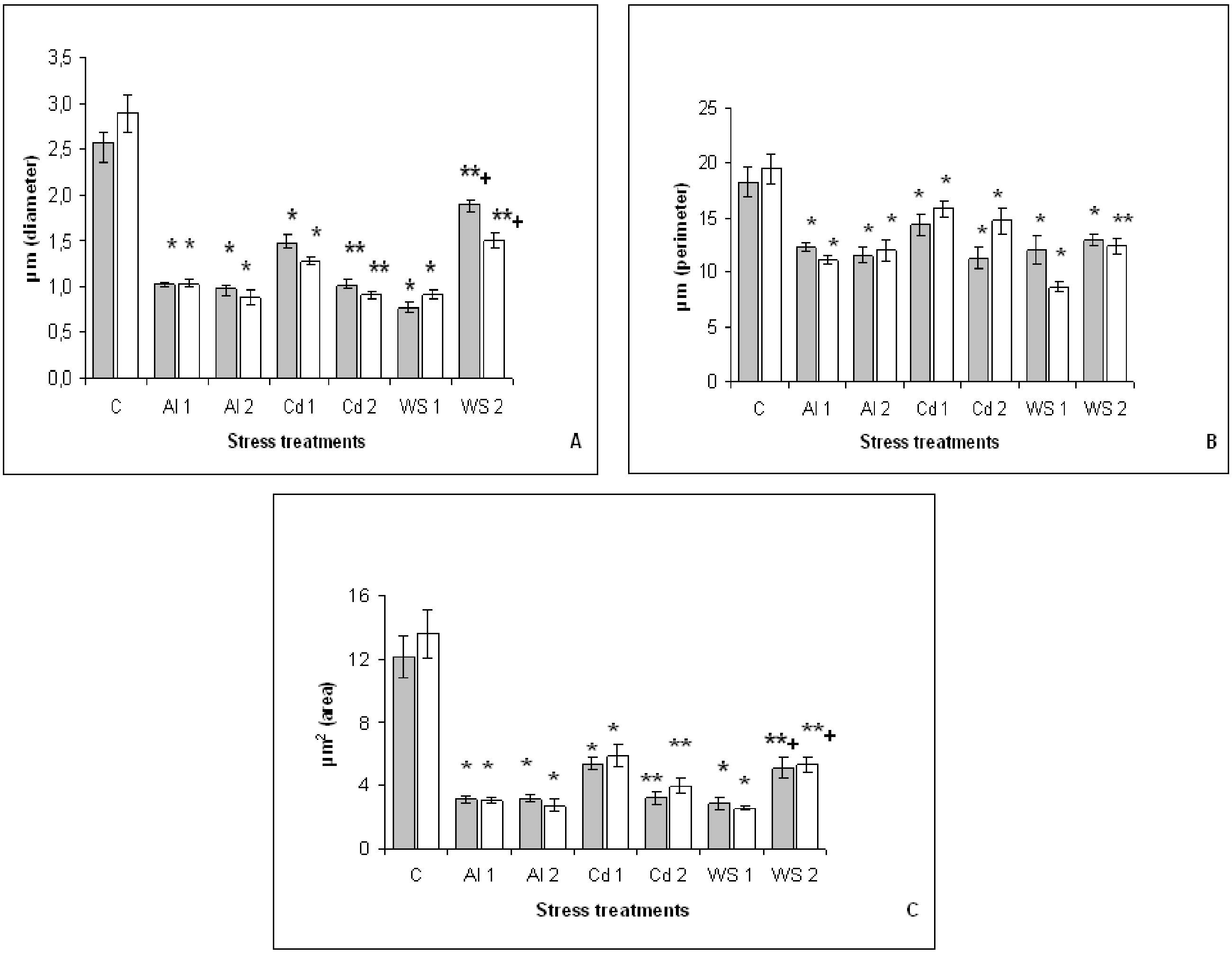

Fig. 2. A-B-C. The effects of different stress factors on stomatal parameters and results of SPSS analyses (A: Diameter, B: Perimeter and C: Area). Left columns (Grey) = Main stem, Right columns (White) = First Lateral Branch

*: significantly different from C, **: significantly different from Cd 1 and C, **+: significantly different from WS 1 and C
*: significantly different from C, **: significantly different from WS 1 and C
**: significantly different from Cd 1 and C, **+: significantly different from WS 1 and C

eters (70.3% reduction in perimeters) and in first lateral branch leaves (81.20% reduction in areas). These results suggest that the WS 1 treated plants showed more sensitivity to severely stressed ones. Despite the fact that closure of stomata is a very effective protection for plants exposed to severe stress levels (Fitter and Hay, 1978) the stomatal diameters and areas under WS 2 treatment were not affected as much as under WS 1 and the reductions in stomatal parameters for WS 2 were significantly different from both WS 1 treatment and controls. The values for WS 2 suggested that this stress level induced the critical leaf water potential. The stomatal aperture begins to narrow, and closure can be complete within 0.5 MPa of the threshold, causing cessation of CO_2 uptake for photosynthesis and stomatal transpiration (Hsiao, 1973) in WS 1. The other resistive mechanism of WS 2 treated plants might be related with clustering of hairs round stomatal pores, which can increase stomatal resistance to water loss (Akıncı, 1997). On the other hand, developing smaller but more densely distributed stomata (no data obtained in the experiment) is seen as an adaptation in leaves growing under conditions of water deficiency, which allows a leaf to reduce transpiration by regulating stomatal mechanisms more rapidly (Hsiao, 1973; Larcher, 1995).

Conclusion

Environmental factors affect whole plants; however, it is the effects on the aerial parts that are most markedly visible. In leaves, guard cell regulation has become an important model system for understanding the regulatory signals that govern stomatal behavior (Comstock, 2002). Stomatal responses have been measured under stress factors such as salinity and drought however, investigations on the influence of heavy metals toxicity on stomatal regulation, especially the effects Al and Cd, were limited.

Al toxicity in molecular terms is still poorly understood (Rengel, 1992; Delhaize and Ryan, 1995). However,

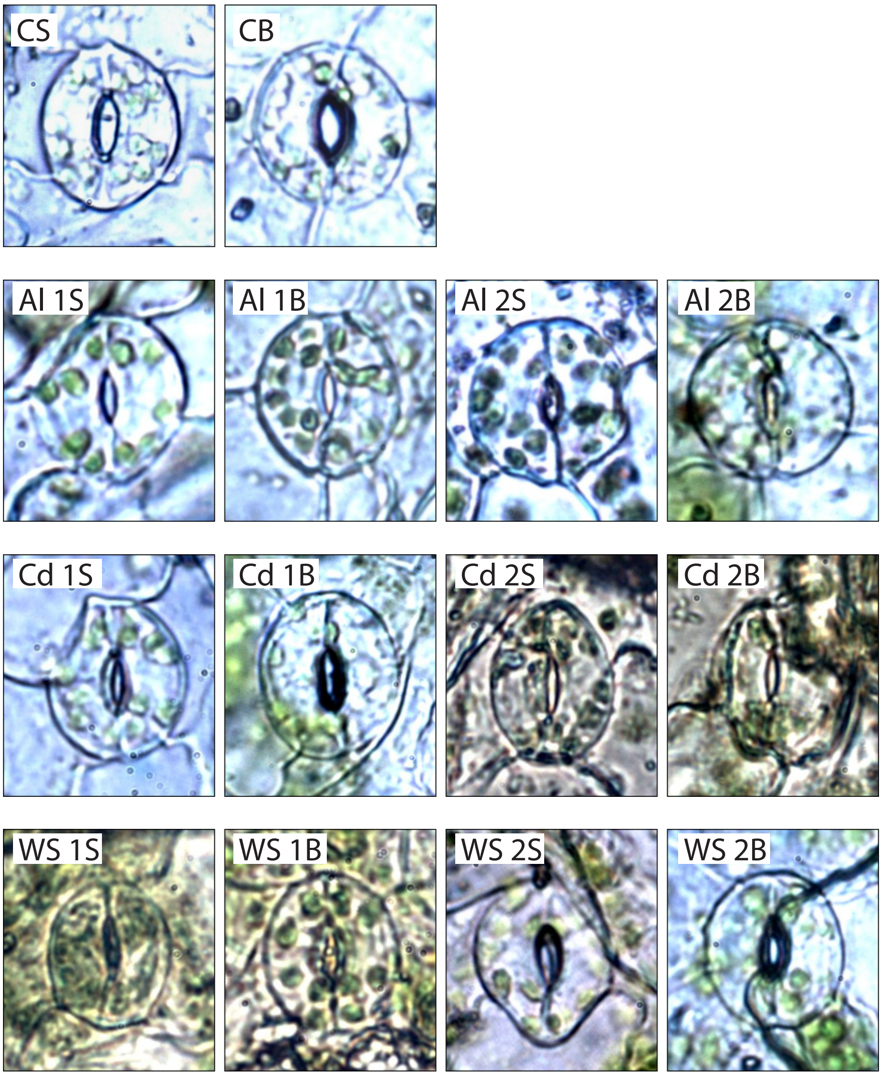

Fig. 3. Stomatal guard cells of 2 months stress treated (Al, Cd and WS) Roman nettle (*Urtica pilulifera* L.). C= Control, S= Main Stem (Leaf), B= First Lateral Branch (Leaf) Al 1= 100µM $AlCl_3$ Al 2= 200µM $AlCl_3$ Cd 1= 100µM $CdCl_2$ Cd 2= 200µM $CdCl_2$ WS 1= Moderate water stress WS 2= Severe water stress. Bars = 5 µm.

common effects include: decrease in total leaf number and size, chlorosis and necrosis of leaves leading to decreased photosynthetic activity (Thornton *et al*., 1986; Kochian, 1995; Jones and Kochian, 1995) and reducing stomatal aperture (Rengel, 1992; Kochian, 1995; Delhaize and Ryan, 1995; Vardar and Ünal, 2007).

Cadmium toxicity on plants requires intensive studies, although some have been undertaken so far, providing some evidence that it affects water balance of plants by reducing root growth, thereby limiting water uptake, and causing partial stomatal closure (Barcelo and Poschenrieder, 1990; Prasad, 1995).

It is clearly known that plants exposed to short-term water deficit respond by reducing stomatal conductance and water loss (Jones and Famjul, 1982; Morgan 1980; Buckley, 2005) and field and greenhouse research indicate that races may respond differently to water stress (Rajakaruna *et al*., 2003). The stomata normally close to reduce water loss from the leaves under drought conditions (Van Iersel and Nemali, 2004). Compared with control values, the stomatal perimeter, diameter and area values were significantly reduced by all treatments of Al, Cd and water stress in both main stem and first lateral branch leaves. The reduction in area and diameter was conspicuous; however, the smaller reduction in perimeter suggests that stomatal closure is accompanied by decreasing stomatal sizes depending on a reduction in epidermal tissues. The data from first lateral branch leaves showed less sensivity to the stress factors than those in main stems, compare to controls. However, the appliance of 100 and 200 µM Al and severe water stress (SS) showed a rather decrease in stomatal measurements in Roman nettle's first lateral branches, whereas 100 and 200 µM cadmium and moderate water stress (WS 1) caused fluctuations of the measured parameters in both main stems and first lateral branches. The results and those of similar studies prove that many stress factors effect hormonal changes (ABA). As a conclusion, stomatal pores are forced to close, as well as effects on different structural and ultrastructural mechanisms of the stomata (Tal and Imber, 1970; Grant and Vatnick, 1998; Buckley, 2005) and other factors, such as leaf position on the main stem or branches.

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References

Adedeji, O. and O. A. Jewoola (2008). Importance of leaf epidermal characters in the *Asteraceae* Family. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 36(2):7-16.

Akgüç, N., İ. İ. Özyiğit and C. Yarcı (2008). *Pyracantha coccinea* Roem. (*Rosaceae*) as a biomonitor for Cd, Pb and Zn in Mugla Province (Turkey). Pakistan Journal of Botany. 40(4):1767-1776.

Akıncı, S. (1997). Physiological responses to water stress by *Cucumis sativus* L. and related species. PhD thesis. University of Sheffield. Department of Animal and Plant Sciences. England.

Barcelo, J. and C. Poschenrieder (1990). Plant water relations as affected by heavy metal stress: a review. Journal of Plant Nutrition. 13(1):1-37.

Barker, J. (2001). The Medicinal Flora of Britain and Northwestern Europe. Winter Press. West Wickham. Kent. UK.

Baytop, T. (1999). Türkiye'de Tıbbi Bitkiler ile Tedavi (Geçmişte ve Bugün). Nobel Tıp Kitabevleri. İstanbul. 232.

Blum, A. (1989). Breeding methods for drought resistance. p. 197-215. In: Jones HG, Flowers TJ, Jones MB (eds). Plants under stress. Biochemistry, Physiology and Ecology and their Application to Plant Improvement. Cambridge University Press. Cambridge.

Bond, W., G. Davies and R. Turner (2006). The biology and non-chemical control of Common Nettle (*Urtica dioica* L.). 1-5. [Online] Available at http://www.gardenorganic.org.uk/organicweeds.

Boyer, J. S. (1982). Plant productivity and environment. Science. 218:443-448.

Buckley, T. N. (2005). The control of stomata by water balance. New Phytologist. 168:275-292.

Comstock, J. P. (2002). Hydraulic and chemical signaling in the control of stomatal conductance and transpiration. Journal of Experimental Botany. 53:195-200.

Cornic, G. J., L. Le Gouallec, J. M. Briantais and M. Hodges (1989). Effect of dehydration and high light on photosynthesis of two C3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f.). Planta. 177(1):84-90.

Das, P., S. Samantaray and G. R. Rout (1997). Studies on cadmium toxicity in plants: a review. Environmental Pollution. 98:29-36.

Davies, P. J. (1987). Plant Hormones and Their Role in Plant Growth and Development. Springer Publishers.

Davies, P. J. (1995). The plant hormone concept: concentration, sensitivity and transport p. 13-38. In: Davies, PJ. (Eds), Plant Hormones. Kluwer Academic Publishers. Dordrecht-Boston-London.

Davies, W. J. and J. Zhang (1991). Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Physiology and Plant Molecular Biology. 42:55-76.

Davis, P. H. (1982). Flora of Turkey and the East Aegean Islands. vol. 7. University Press. Edinburgh. 633-636.

Delhaize, E. and P. R. Ryan (1995). Aluminum toxicity and tolerance in plants. Plant Physiology. 107:315-321.

Epstein, E. and W. J. Grant (1973). Water stress relations of the potato Bull. 843. Plant under field conditions. Agronomy Journal. 65:400-404.

Fatemy, F., P. K. E. Trinder, J. N. Wingfiel and K. Evans (1985). Effects of *Globodera rostochiensis*, water stress and exogenous abscisic acid on stomatal function and water use of Cara and Pentland Dell potato plants. Revue Nématology. 8(3):249-255.

Firbank, L. G., L. R. Norton and S. M. Smart (2002). Recording cereal field margins in Countryside Survey 2000. Report to the Department for Environment. Food and Rural Affairs 16.

Fitter, A. H. and R. K. M. Hay (1978). Environmental Physiology of Plants. Academic Press. New York.

Garbutt, K., W. E. Williams and F. A. Bazzaz (1990). Analysis of differential response of five annuals to elevated CO_2 during growth. Ecology. 71(3):1185-1194.

Grant, B. W. and I. Vatnick (1998). A multi-week inquiry for an undergraduate introductory biology laboratory: Investigating correlations between environmental variables and leaf stomata density. Journal of College Science Teaching. 28:109-112.

Grime, J. P., J. G. Hodgson and R. Hunt (1988). Comparative Plant Ecology. Unwin Hyman Ltd. London. UK.

Hsiao, T. C., J. C. O'Toole, E. B. Yambao and N. C. Turner (1984). Influence of osmotic adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L.). Plant Physiology. 75(2):338-341.

Hsiao, T. C. (1973). Plant response to water stress. Annual Review of Plant Physiology. 24:519-570.

Hoagland, D. R. and D. I. Arnon (1950). The water culture method for growing plants without soil. Circular 347. Agricultural Experimental Station. University of California. Berkeley.

Huang, C. Y., F. A. Bazzaz and L. N. Vanderhoef (1974). The Inhibition of Soybean Metabolism by Cadmium and Lead. Plant Physiology. 54:122-124.

Huber, S. C., H. H. Rogers and F. L. Mowry (1984). Effects of water stress on photosynthesis and carbon partitioning in soybean (*Glycine max* [L.] Merr.). Plant Physiology. 76(1):244-249.

Jones, D. L. and L. V. Kochian (1995). Aluminum inhibition of the inositol 1-4-5-trisphosphate signal transduction pathway in wheat roots: A role in aluminum toxicity? Plant Cell. 7:1913-1922.

Jones, H. G. (1992). Plants and microclimate: a quantitative approach to environmental plant physiology. Cambridge University Press. New York. 51.

Jones, H. G. and L. Famjul (1982). Effects of water stress on CO_2 exchange in Apple. Effects of stress on photosynthesis. In: Proceedings of a conference hels at the "Limburgs Universtair Centrum" Diepenbeek. Belgium. 75-84.

Kavalalı, G., H. Tuncel, S. Göksel and H. H. Hatemi (2003). Hypoglycemic activity of *Urtica pilulifera* in streptozotocin-diabetic rats. Journal of Ethnopharmacology. 84:241-245.

Kochian, L. V. (1995). Cellular mechanisms of aluminum toxicity and resistance in plants. Annual Review of Plant Physiology and Plant Molecular Biology. 46:237-260.

Kosma, D. K., J. A. Long and S. D. Ebbs (2004). Cadmium Bioaccumulation in Yellow Foxtail (*Setaria glauca* L. P. Beauv): Impact on Seed Head Morphology. American Journal of Undergraduate Research. 3:9-14.

Kozlowski, T. T. (1997). Responses of woody plants to flooding and salinity. Tree Physiology Monograph. 1:1-29.

Larcher, W. (1995). Physiological Plant Ecology. Ecophysiology and stress physiology of functional groups. Springer. Berlin.

Leyman, B., D. Gelen, F. J. Quintero, M. R. Blatt (1999). A tobacco syntaxin with a role in hormonal control of guard cell ion channels. Science. 283(5401):537-540.

Matsumoto, H. (2000). Cell biology of aluminum toxicity and tolerance in higher plants. International Review of Cytology. 200:1-46.

Morgan, J. M. (1980). Differences in adaptation to water stress within crop species. Adaptation of plants to water and high temperature stress, p. 369-382. In: Turner NC and Kramer PJ (eds). John Wiley and Sons. New York.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant, Cell & Environment. 25:239-250.

Neil, S. H. and J. T. Gregory (2001). Remobilization of cadmium in maturing shoots of near isogenic lines of durum wheat that differ in grain cadmium accumulation. Journal of Experimental Botany. 52:1473-1481.

Nocito, F. F., L. Pirovano, M. Cocucci and G. A. Sacchi (2002). Cadmium-induced sulfate uptake in maize roots. Plant Physiology. 129(4):1872-1879.

Paquin, R. and G. R. Mehuys (1980). Influence of soil moisture on cold tolerance of alfalfa. Canadian Journal of Plant Science. 60:139-147.

Parker, J. (1968). Drought-resistance mechanisms, p. 195-234. In: Kozlowsky TT (eds), Water deficits and plant growth. Academic Press. New York.

Prasad, M. N. V. (1995). Cadmium toxicity and tolerance in vascular plants. Environmental and Experimental Botany. 35:525-545.

Quick, W. P., M. M. Chaves, R. Wendler, M. David, M. L. Rodrigues, J. A. Passaharinho, J. S. Pereira, M. D. Adcock, R. C. Leegood and M. Stitt (1992). The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. Plant. Cell and Environment. 15:25-35.

Rajakaruna, N., G. E. Bradfield, B. A. Bohm and J. Whitton (2003). Adaptive differentiation in response to water stress by edaphic races of *Lasthenia californica* (*Asteraceae*). International Journal of Plant Sciences. 164:371-76.

Rambal, S. and G. Debussche (1995). Water balance of Mediterranean ecosystems under a changing climate. Global change and Mediterranean-type ecosystems, p. 386-407. In: Morene JM. Oechel WC (Eds). Ecologic studies. Vol. 117. Springer-Verlag. New York.

Raschke, K. and A. Resemann (1986). The midday depression of CO_2 assimilation in leaves of *Arbutus unedo* L.: diurnal changes in photosynthetic capacity related to changes in temperature and humidity. Planta. 168(4):546-558.

Rengel, Z. (1992). Role of calcium in aluminum toxicity. New Phytologist. 121:499-513.

Richter, H. and S. B. Wagner (1982). Water stress resistance of photosynthesis: Some aspects of osmotic relations. Effects of stress on photosynthesis. Proceedings of a conference held at the "Limburgs Universitair Centrum" Diepenbeek. Belgium. 45-53.

Salisbury, E. (1962). The biology of garden weeds. Part II. Journal of the Royal Horticultural Society. 87:458-470 and 497-508.

Sandalio, L. M., H. C. Dalurzo, M. Gomez and M. C. del Rio LA. Romero-Puertas (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. Journal of Experimental Botany. 52(364):2115-2126.

Sayed, S. A. (1997). Effect of cadmium and kinetin on transpiration rate, stomatal opening and leaf relative water content in safflower plants. Journal of Islamic Academy of Sciences. 10(3): 73-80.

Schroeder, J. I. (1988). Potassium transport properties of potassium channels in the plasma membrane of *Vicia faba* guard cells. The Journal of General Physiology. 92:667-684.

Schroeder, J. I., J. M. Ward and W. Gassmann (1994). Perspectives on the physiology and structure of inward-rectifying K-channels in higher plants. Biophysical implications for

K-uptake. Annual Review of Biophysics & Biomolecular Structure. 23:441-471.

Sharp, R. E. and W. J. Davies (1979). Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta. 147(1):43-49.

Schulze, E. D. (1986). Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. Annual Review of Plant Physiology. 37:247-274.

Shekharv, C. and M. Iritani (1979). Influence of moisture stress during growth on $14CO_2$ fixation and translocation in *Solanum tuberosum* L. American Potato Journal. 56:307-311.

Sivaguru, M., B. Ezaki, Z. H. He, H. Tong, H. Osawa, F. Baluska, D. Volkmann and H. Matsumoto (2003). Aluminum-induced gene expression and protein localization of a cell wall-associated receptor kinase in *Arabidopsis*. Plant Physiology. 132(4):2256-66.

Tal, M. and D. Imber (1970). Abnormal stomatal behavior and hormonal imbalance in flacca, a wilty mutant of tomato. Plant Physiology. 46:373-376.

Tardieu, F. and W. J. Davies (1993). Root-shoot communication and whole-plant regulation of water flux, p. 147-162. In: Smith, J. A. C., Griffiths, H. (eds), Water Deficits. Plant Responses from Cell to Community. Bios Scientific Publishers. Oxford.

Thornton, F. C., M. Schaedle and D. L. Raynal (1986). Effect of aluminum on the growth of sugar maple in solution culture. Canadian Journal of Forest Research. 16:892-896.

Van Iersel, M. W. and K. S. Nemali (2004). Drought stress can produce small but not compact marigolds. Horticultural Science. 39(6):1298-1301.

Vardar, F., E. Arıcan and N. Gözükırmızı (2006). Effects of aluminum on *in vitro* root growth and seed germination of tobacco (*Nicotiana tabacum*). Advances in Food Sciences. 28(2):85-88.

Vardar, F. and M. Ünal (2007). Aluminum toxicity and resistance in higher plants. Advances in Molecular Biology. 1:1-12.

Vitorello, V. A., F. R. C. Capaldi and V. A. Stefanuto (2005). Recent advances in aluminum toxicity and resistance in higher plants. Brazilian Journal of Plant Physiology. 17:129-143.

Wagner, G. J. (1993). Accumulation of cadmium in crop plants and its consequences to human health. Advances in Agronomy. 51:173-212.

Wilman, D. and J. A. Riley (1993). Potential nutritive value of a wide range of grassland species. Journal of Agricultural Science. 120:43-49.

Wong, S. C., I. R. Cowan and G. D. Farquhar (1985). Leaf conductance in relation to rate of CO_2 assimilation III. Influences of water stress and photoinhibition. Plant Physiology. 78(4):830-834.

Wright, S. T. C. (1969). An increase in the Inhibitor-B content of detached wheat leaves following a period of wilting. Planta Berlin. 86:10-20.

Wright, S. T. C. and R. W. P. Hiron (1969). Abscisic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. Nature London. 224:719-720.

Zelitch, I. (1971). Photosynthesis, Photorespiration and Plant Productivity. Academic Press. New York. 1-347.