A REVIEW ON SAMPLING XYLEM SAP

Ubirajara Contro Malavasi[1]; Marlene de Matos Malavasi[1]; Ingela Stadenberg[2]

[1]UNIOESTE-Centro de Ciências Agrárias, Rua Pernambuco 1777, CEP: 85960-000, Mal. Cândido Rondon-PR, Brazil. e-mail: umala@unioeste.br

[2]Swedish University of Agricultural Sciences, P.O. Box 7070, SE-750 07, Uppsala, Sweden. e-mail: ingela.stadenberg@ slu.se

ABSTRACT: This review presents and discusses recent methodologies used mainly by European researchers to study and to evaluate fluctuations and composition of xylem sap collected from plant leaves in vivo. Methods such as root pressure exudation, Scholander Pressure Chamber, xylem pressure probe refined method, root chamber pressure technique are crucial to study the dynamic variations of xylem sap composition and nutrient fluxes, Considerations on the temporal and spatial variation of sap fluxes and the controversy involved in these measurements are discussed in the paper.

Key words: attached leaves, xylem sap sampling, xylem sap composition, xylem sap measurements

UMA REVISÃO SOBRE A AMOSTRAGEM DE SEIVA XILEMÁTICA

RESUMO: Esta revisão apresenta e discute metodologias recentes utilizadas principalmente por pesquisadores europeus para estudar e avaliar as flutuações e composição da seiva do xilema amostradas de folhas vegetais *in vivo*. Métodos como a exudação por pressão na raiz, a bomba de Scholander, o método refinado do sensor de pressão xilemático, a técnica do recipiente sob pressão são cruciais para estudar a dinâmica das variações na composição e nos fluxos de nutrientes. Considerações sobre a variação espaço-temporal dos fluxos da seiva são mencionados além das polêmicas envolvendo estas mensurações.

Palavras-chave: folhas intactas, amostragem da seiva xilemática, composição da seiva xilemática, avaliações da seiva xilemática

INTRODUCTION

Water flow through the soil-plants-atmosphere continuum is one of the most important factors in the biosphere-atmosphere interactions. This process involves several physical, chemical, and biological aspects. Water moves into roots driven by a hydraulic water flow across the apoplast and a parallel flow across cells which bears both hydraulic and osmotic components (Steudle and Peterson, 1998). The relative importance of tension (negative pressure) created by transpiration and the diffusive transport of solutes which couples to water flow is still under discussion. However, it is agreed that interactions between solute and water flow in transpiring plants will not be too important since the hydrostatic gradient will usually be the dominating force (Steudle, 2000).

Nowadays, there is increasing evidence that the water balance can also be regulated at the input, i.e. by changing the capacity of roots to take up water (Steudle, 2000, 2001). In short term, water content may be regulated by mechanisms that change the physical properties of roots, i.e. the switching between cellular and apoplastic pathways (Steudle, 2001) or by a gating of water channels (aquaporins) of root cells which may change in a diurnal rhythm (Henzler et al., 1999; Tyerman et al., 1999).

Xylem transport is crucial for nutrient acquisition by plants, as well as the dominant pathway for mediating signals that control interaction between plant parts. Plant nutrient cycling bears important roles in both nutrition-related and regulatory processes. Nutrient transport from root to leaves is a dynamic and complex process. Non - destructive methodological developments during recent years provide the basis for temporal and spatial resolution sufficient to study actual fluxes of nutrients and signals of plants and will lead to a better understanding of reasons and consequences of such dynamic processes.

There have been many reports of diurnal

variation in nutrient uptake (Clement et al., 1978; Hansen 1980; Delhon et al., 1995a,b). Since nutrient uptake depends on active and passive processes, energy limitation can be relevant to diurnal variation not only on nutrient uptake but also on nutrient transport. For example, abscisic acid may be a hormonal stress signal that moves in the xylem from the root to the different parts of the shoot where it regulates transpiration water loss and leaf growth (Hartung et al., 2002).

Drought is an important factor limiting the distribution and growth of plants, which possess various morphological and physiological adaptations that assist them in avoiding or tolerating desiccation. Over the last two decades, reports on stomatal closure before a change in leaf water status have been attributed to the transport of a chemical signal (likely abscisic acid, ABA) from roots to shoots in the transpiration stream (Steudle, 2000). In the absence of leaf dehydration, the site of synthesis of the ABA translocated in the xylem sap of plants during drought is presumably the roots (root-originated ABA) (Zhang et al., 1987). In plants where both roots and leaves dehydrate during drought, xylem sap may contain a combination of root-originated ABA and ABA redistributed to roots from the leaves into the phloem (root-sourced ABA) (Loveys, 1984; Wolf et al., 1990; Liang et al., 1997). Recent data suggest that roots have a remarkable capacity to alter their water permeability over short term in response to day and night cycles, nutrient deficiency, or stress. Such capacity can be mostly accounted for by changes in cell permeability mediated by aquaporins (Ye et al., 2004).

SAMPLING TECHNIQUES

Discussions about the validity of the cohesion/tension mechanism are not new. Adequate methods to sample xylem sap are crucial to study the dynamic variations of xylem sap composition and nutrient fluxes. Since most researches are mainly interested in the nutrient dynamics under natural conditions, the basic requirement of a technique is not to interfere with the forces and processes that govern nutrient fluxes within intact plants. Common techniques such as root pressure exudation and the Scholander pressure chamber methods require action being destructive to the plant system and, consequently, may impose risks of severe interference with relevant plant processes, which determine nutrient fluxes in intact plants. Such statement, reminds the users of the limitations of methods.

One main difference between root pressure exudates and xylem sap from intact plants is that flux rates at which these solutions have been sampled differ significantly. Usually, the exudation rates are in the range of a few microliters per minute whereas fluxes in intact plants across the same cross-section can be higher by as much as 100 times (Schurr and Schulze, 1995). This can cause differences in the dilution of the ions loaded into the xylem by active transport processes in the root. Application of pneumatic pressure to the root system can increase the flux of transpiration-like ratios (i.e. outflow of water driven by physical forces). Concentration of exudates under these conditions is in the range of saps from intact plants although the relative abundance of various ions is variable due to impact of the sampling method upon xylem loading (Schurr and Schulze, 1995).

The Scholander pressure method has been criticized over the years due to the risk in changing the composition of the sap in the xylem after the excision of the plant part by both water and ion fluxes (Zimmermann et al., 1994; Schurr, 1998). It is clear that results obtained with this technique have to be interpreted with care especially under field conditions. More recently, Borel and Simonneau (2002) compared low-volume extracts (less than 0.35 mm^3 cm^{-2} leaf area) collected from leaves of well-watered plants from several lines of *N. plumbaginifolia*. The above authors showed that $[ABA]_{sap}$ was close to $[ABA]_{solution}$ obtained by feeding leaves with known ABA concentration from 0–500 μmol m^{-3} for 2–3 h before collection demonstrating that determination from a low-volume sample was quite reliable.

The xylem-pressure probe method for sampling of xylem sap allows individual xylem vessels to be punctured by a capillary glass (Balling and Zimmermannn, 1990). The capillary is pushed into the tissue until a negative pressure indicates that positioning in a functional xylem vessel has been achieved. For actual sap sampling, a negative pressure is compensated by applying pressure to the root system (Zimmermann et al., 1995). The strength of this technique is its similarity to the transport of nutrients in the xylem, specially if very small volumes of samples are obtained (Bazzanella et al., 1997), and to the localization of stable isotopes (Kuhn et al., 1995). Its application in studies of ion balance is limited however by the susceptibility of the xylem to cavitation during sampling. Because of the reported differences in individual xylem vessels (Schurr, 1999), it is necessary to collect very small samples to obtain a mean value relevant for calculation of ion balance for the entire plant organ.

Steudle and Peterson (1998) reported success with measurements of xylem pressure using a set-up (Figure 1) which yielded immediate response changes in transpiration caused by an increase in light intensity.

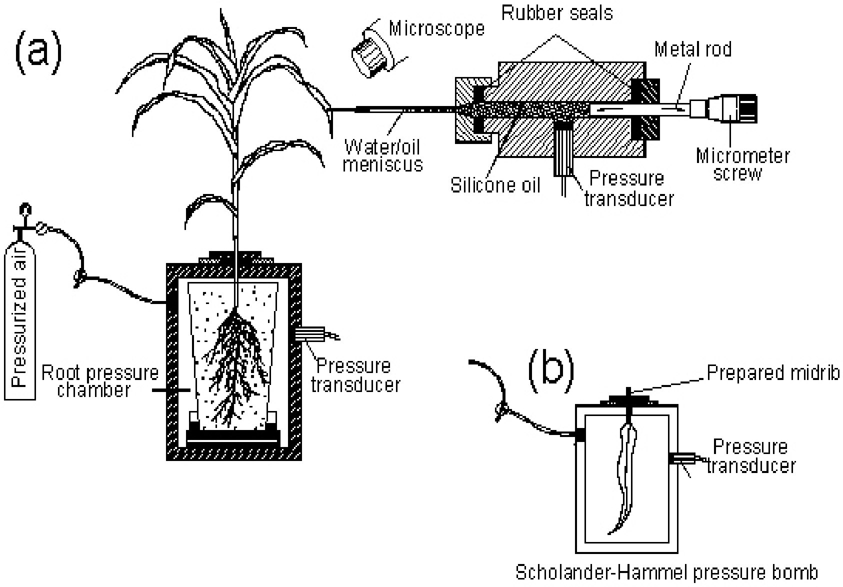

Figure 1 - Xylem sap techniques: (a) a four-week-old maize root placed in a root pressure chamber allowing leaf xylem pressure (P_x) to be changed either by altering pneumatic pressure (P_g) within the root chamber, or by changing illumination. P_x is measured with a cell pressure probe filled with silicone oil except for the tip, which is filled with water; (b) a Scholander-Hammel pressure chamber which measures xylem pressure in the leaf after the P_x has been measured with the pressure probe.

The root chamber pressure technique allows xylem sap to be sampled continuously from intact transpiring plants, a condition which is essential to study the dynamics of nutrient transport in the xylem. However, it is solely a laboratory method since it requires sealing the entire root system inside a pressure chamber (McDonald and Davies, 1996). Xylem sap is obtained by application of pneumatic pressure at the root system. This can be accomplished over a period of several days without alteration of gas exchange, growth and phloem transport (Heckenberg et al., 1998). The limitation of the system to soil-grown plants was eliminated recently with the development of a spray-pressure chamber (Herdel et al., 2001). In this modified system, nutrient solution is supplied by nozzles to the roots hanging freely in the gaseous atmosphere similar to aeroponic culture. The root-pressure technique is presently recommended for a range of tensions (up to 10 bars). A recent comparison (Goodger et al., 2005) using three techniques in corn (plants detopped by severing the mesocotyl 0.5 cm below the shoot and xylem sap collected from the cut surface of the mesocotyl under 'root pressure'; plants detopped 0.5 cm below the shoot and each pot containing the roots and exposed mesocotyl placed in custom-built pressure vessels; and plants sealed in the pressure vessels around the mesocotyl without detopping the shoots) revealed that concentrations of anions, cations, and organic acids in the sap were significantly different between the three extraction techniques and produced different sap flow rates.

TEMPORAL AND SPATIAL VARIATION OF SAP FLUXES

Strong diurnal variations in xylem sap composition (cations, anions, amino acids and pH) have been reported in plants such as sunflower, castor bean and poplar using the root-pressure chamber (Gollan et al., 1992; Schurr et al., 1992; Schurr and Shulze, 1995, 1996). In these reports, diurnal variations were much larger than those obtained by the mean values with stress treatments. The range of diurnal variation in pH was closely related to variation in drought stress as shown by Schurr and Schulze (1996). Also, diurnal changes in xylem sap composition have been found to occur with different sampling methods and species (Andersen et al., 1993, 1995), including tropical trees (Barker and Becker, 1995). Coupling sap flow velocity and nitrogenous compounds translocated in the xylem sap has been suggested as a means to estimate nutrient fluxes to new shoots during spring growth in field-growing trees (Millard et al., 1998).

Diurnal variation were also recorded for the analyzed substances which has been suggested to fit three categories: one in which the course of concentration varied inversely with the transpiration rate over the whole day; a second showing high concentration during the night and lower concentration during the day with concentration varying during the light period despite constant transpiration; and the last in which concentrations changed proportionally to transpiration (Schurr and Schulze, 1995).

More recent studies showed that the amplitude and diurnal pattern of individual nutrients are very dependent on the availability of the individual nutrient to the roots (Heckenberg et al., 1998). Nutrient mass fluxes are dependent on the relation between concentration in the xylem sap and the flux rate of the xylem. However, it has been kept in mind that additional pathways for ion transport can contribute to the distribution of ions in the plant.

In recent years, there has been increased interest in the processes that underline regulation of root water transport in response to environmental factors. Water channel proteins named "aquaporins" found in the membrane of root cells are in part responsible for mediating those processes (Javot and Maurel, 2002). Since their discovery, more and more studies demonstrated that water channels represent the main selective pathway for water to move through the membranes of both plant and animal cells. Lu and Neumann (1999) reported that in rice seedlings the application of mercurials had an effect on whole plant conductance under water stressed conditions. The authors' interpretation indicated that apoplastic water transport predominated in normal conditions and that water channels had been up-regulated in water-stress conditions.

Ye et al. (2004) have demonstrated that gating of water channels in *Chara* internodes by high concentration does occur. According to the proposed model, tensions within water channels (aquaporins) would affect the open / closed state by changing the free energy between states favoring a distorted/ collapsed rather than the open state. The same authors argued that the gating of water channels could play an important role in regulating water transport across cell membranes. Furthermore, their experimental work showed that the activity of water channels could be decreased by the concentration and size of solutes. Therefore, the bigger the solute sizes were the lower the required concentration was to induce a reversible closure of aquaporins.

Ranathunge et al. (2004) working with mature (50-100 mm) and immature (20-50 mm) rice root segments from the apex concluded the existence of a predominantly apoplastic water flow across the outer part of roots in spite of the presence of apoplastic barriers such as Casparian strips (suberin bands) of the exodermis.

Even in forest ecosystems there exists a wide variation among species on water used. Xylem sap flow measurements indicated that the daily sap flow of *Carapa* sp. (3.8 l day^{-1} tree^{-1} to 16.4 l day^{-1} tree^{-1}) exceeded the daily sap flow of *Swietenia* sp. (2.4 l day^{-1} tree^{-1} to 7.0 l day^{-1} tree^{-1}) and of *Cedrela* sp. (1.6 l day^{-1} tree^{-1} to 11.6 l day^{-1} tree^{-1}) during the entire year in the Amazon region (Dünisch and Morais, 2002).

PROCESSES POTENTIALLY INVOLVED IN TEMPORAL VARIATION

The processes which contribute to temporal variation in xylem sap are still blurry inspite of the list of possible ones. Temporal and spatial variations are most likely to be normal than a constant xylem sap concentration because plants have to cope with them. Nutrient availability to roots can vary in time and space due various processes in the soil (Nye and Tionker, 1977, Cameron and Haynes, 1986; Marschner, 1995). Due to the variation in the pattern of nutrient transport in the soil and nutrient culture, it seems to be unlikely that they are due to changes in nutrient availability for plants to uptake. Consequently, the availability of nutrients is more likely to be involved in the amplitude of diurnal nutrient dynamics (Herdel et al., 2001).

Root pressure chamber and transpiration measurements of the shoot enable a continuous analysis of xylem transport with a high temporal resolution (Schurr, 1998; Herdel et al., 2001). In addition, use of several sampling sites can facilitate to monitor spatial resolution. Consequently, we foresee that a combination of such approach with nutrient uptake experiments and tracer methods has a high potential for further understanding the dynamics of xylem sap transport on a whole-plant basis.

CONCLUSION

During the 90s, a number of researchers, including Martin Canny (Carleton University, Ottawa, Canada), Ulrich Zimmermann (University of Wuerzburg, Germany), and Rick Meinzer (USDA Forest Service , Oregon, USA) challenged the validity of the cohesion-tension hypothesis of water transport. The above mentioned scientists argued that pressure measurements in xylem using the pressure probe and the pressure chamber often revealed tensions far smaller (i.e. pressure potential closer to zero) than would be predicted if the cohesion-tension hypothesis was in fully operation.

These challenges spurred a lively debate, with a number of world-renowned water relations scientists (Melvin Tyree, John Sperry, E. Steudle, Will Pockman, and "Missy" Holbrook) piling on. The resulting body of literature not only helped to advance the field of plant (specially tree) water relations forward, but also provided a fascinating study on the role of the dogma and the scientific method in establishing the paradigms of the field.

The technique using detopped plants (by severing the mesocotyl 0.5 cm below the shoot) and xylem sap collected from the cut surface of the mesocotyl under root pressure has the advantage of being a simple means of extracting large volumes of sap, but on the other hand, it does not provide a quantitative estimate of sap constituent delivery rates. Pressurization techniques enable the accurate estimation of delivery rates of most sap constituents if pressure is applied to match whole plant transpiration. In addition, pressurization also enables the extraction of sufficient sap volumes from leaves which in turn determines whether sap constituent modification occurs from the roots to the leaves.

Water in the xylem is under tension. At low pressures, a liquid is vulnerable to cavitation and formation of gas bubbles which decreases hydraulic conductivity and may lead to irreversible embolism of conduits, thus harmful for plants. The understanding of sap ascent does not end here. There exists controversial ideas on the cavitation processes and the ways plants possibly repair embolism. A better understanding on the flow at scale of tracheids is required and measurement techniques for flow and cavitation rates must be improved.

REFERENCES

ANDERSEN, P. C.; BRODBECK, B. V.; MIZELL, R. F. Diurnal variation in tension, osmolarity, and the composition of nitrogen and carbon assimilates in xylem fluid of *Prunus persica*, *Vitis* hybrid, and *Pyrus communis*. **Journal American Society of Horticulture Science**, Madison, n. 120, p. 600-606, 1995.

_______. Diurnal variation of amino acids and organic acids in xylem fluid from *Lagerstroemia indica*: an endogenous circadian rhythm. **Physiology Plantarum**, n. 89, p. 783-790, 1993.

BALLING, A.; ZIMMERMANN, U. Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. **Planta,** n. 182, p. 325-338, 1990.

BARKER, M.; BECKER, P. Sap flow rate and sap nutrient content of a tropical rain forest canopy species, *Drybalanops aromatica*, in Brunei. **Selbyana,** n. 16, p. 201-211, 1995.

BAZZANELLA, A. et al. Determination of inorganic anions, carboxylic acids and amino acids in plant matrices by capillary electrophoresis. **Chromatographia**, n. 45, p. 59-62, 1997.

BOREL, C.; SIMONNEAU, T. Is the ABA concentration in the sap collected by pressuring leaves relevant for analyzing drought effect on stomata? Evidence from ABA-fed leaves of transgenic plants modified capacities to synthesize ABA. **Journal of Experimental Botany**, v. 53, n. 367, p. 287-296, 2002.

CAMERON, K. C.; HAYNES, R. J. Retention and movement of nitrogen in soils. In: HAYNES, R.J. (Ed.). **Mineral nitrogen in the plant-soil system**. Orlando: Academic Press, 1986. p. 127-165.

CLEMENT, C. R et al. The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. II. Effect of light, defoliation, and relationship to CO_2 flux. **Journal of Experimental Botany**, v. 29, p. 1173-1183, 1978.

DELHON, P. et al. Diurnal regulation of NO^-_3 uptake in soybean plants. I. Changes in NO^-_3 influx, efflux, and N utilization in the plant during the day/night cycle. **Journal of Experimental Botany,** v. 46, p. 1585-1594, 1995a.

_______. Diurnal regulation of NO^-_3 uptake in soybean plants. II. Relationship with accumulation of NO^-_3 and asparagine in the roots. **Journal of Experimental Botany,** v. 46, p. 1595-1602, 1995b.

DÜNISCH, O.; MORAIS, R. R. Regulation of xylem sap flow in an evergreen, a semi-deciduous, and a deciduous Meliaceae species from the Amazon**. Trees Structure and Function**, v. 16, n. 6, p. 404-416, 2002.

GOLLAN, T.; SCHURR, U.; SCHULZE, E. D. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids and the pH of the xylem sap. **Plant, Cell and Environment**, n. 15, p. 551-559, 1992.

GOODGER, J. Q. D. et al. Relationships between xylem sap constituents and leaf conductance of well-watered and water-stressed maize across three xylem sap sampling techniques. **Journal of Experimental Botany**, v. 56, n. 419, p. 2389-2400, 2005.

HANSEN, G. K. Diurnal variation of root respiration rates and nitrate uptake as influenced by nitrogen supply. **Physiology Plantarum**, n. 48, p.421-427, 1980.

HARTUNG, W.; SAUTER, A.; HOSE, E. Abscisic acid in the xylem: where does it come from, where does it go to? **Journal of Experimental Botany**, v. 53, n. 366, p. 27-32, 2002.

HECKENBERG, V.; ROGGATZ, U.; SCHURR, U. Effect of drought stress on the cytological status in *Riccinus communis*. **Journal of Experimental Botany**, v. 49, p. 181-189, 1998.

HENZLER, T. et al. Diurnal variation in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonica*. **Planta**, n. 210, p. 50 – 60, 1999.

HERDEL, K. et al. Dynamics of concentration and nutrient fluxes in the xylem of *Riccinus communis* diurnal course, impact of nutrient availability and nutrient uptake. **Plant, Cell and Environment**, n. 24, p. 41-52, 2001.

JAVOT, H.; MAUREL, C. The role of aquaporins in root water uptake. **Annals of Botany**, n. 90, p. 301-313, 2002.

KUHN, A.; BAUCH, J.; SCHRODER, W. H. Monitoring uptake and contents of Mg, Ca and K in Norway spruce as influenced by pH and Al using microprobe analysis and stable isotope labeling. **Plant Soil**, n. 168/169, p. 135-150, 1995.

LIANG, J.; ZHANG, J.; WONG, M. H. How do roots control xylem sap ABA concentration in response to soil drying ? **Plant Cell Physiology**, n. 38, p.10-16, 1997.

LOVEYS, B. R. Abscisic acid transport and metabolism in grapevine (*Vitis vinifera* L.). **New Phytologist**, n. 98, p. 575-582, 1984.

LU, Z.; NEUMANN, P. M. Water stress inhibits hydraulic conductance and leaf growth in rice seedlings but not the transport of water via mercury-sensitive water channels in the root. **Plant Physiology**, n. 120, p. 143-151, 1999.

MARSCHNER, H. **Mineral nutrition of higher plants.** New York: Academic Press, 1995. 889 p.

McDONALD, A. J. S.; DAVIES, W. J. Keeping in touch: responses of whole plant to deficits in water and nitrogen supply. **Advances in Botanical Research**, n. 22, p. 229-300, 1996.

MILLARD, P. et al. Variations in the amino acid composition of xylem sap of *Betula pendula* Roth. trees due to remobilization of stored N in the spring. **Plant, Cell and Environment**, n. 21, p. 715-722, 1996.

NYE, P. H.; TINKER, P. B. **Solute movement in the soil-root system**. Oxford:Blackwell, 1977. 342 p.

RANATHUNGER, K. et al. Water permeability and reflection coefficient of the outer part of young rice roots are differently affected by closure of water channels (aquaporins) or blockage of apoplastic pores. **Journal of Experimental Botany.**, v. 55, p. 443-447, 2004.

SCHURR, U. Dynamics of nutrient transport from the root to the shoot. **Progress in Botany**, n. 60, p. 234-253, 1999.

________. Xylem sap sampling – new aspects and techniques in an old topic. **Trends in Plant Science**, n. 3, p. 293-373, 1998.

________; GOLLAN, T.; SCHULZE, E. D. Stomatal response to drying soil relation to changes in the xylem sap composition of *Helium annus*. II. Stomatal sensitivity to abscisic acid imported fro the xylem sap. **Plant, Cell and Environment**, n.15, p. 561-567, 1992.

_________; SCHULZE, E. D. Effect of drought on nutrient transport and ABA transport in *Riccinus communis*. **Plant, Cell and Environment**, n. 19, p. 665-674, 1996.

_________; SCHULZE, E. D. The concentration of xylem constituents in root exudate, and in sap from intact, transpiring castor bean plants (*Riccinus communis* L.). **Plant, Cell and Environment**, n. 18, p. 409-420, 1995.

STEUDLE, E. Water uptake by plant roots: an integration of views. **Plant and Soil**, n. 226, p. 45-56, 2000.

_________. The cohesion-tension mechanism and the acquision of water by plant roots. **Annual Review of Plant Physiology and Plant Molecular Biology**., n. 52, p. 847 – 875, 2001.

_________; PETERSON, C. A. How does water get through roots? **Journal of Experimental Botany**, v. 49, n. 322, p. 775-788, 1998.

TYERMAN, S. D. et al. Plant acuaporins: their molecular biology, biophysics and significance for plant water relations. **Journal of Experimental Botany**, v. 50, p.1055-1071, 1999.

WOLF, O.; JESCHKE, W. D.; HARTUNG, W. Long-distance transport of abscisic acid in NaCl-treated intact plants of *Lupinus albus*. **Journal of Experimental Botany**, v. 41, p. 593-600, 1990.

YE, Q.; WIERA, B.; STEUDLE, E. A cohesion/tension mechanism explains the gating of water channels (aquaporins) in *Chara* internodes by high concentration. **Journal of Experimental Botany**, v. 55, p. 449-461, 2004.

ZHANG, J.; SCHURR, U.; DAVIES, W. J. Control of stomatal behavior by abscisic acid which apparently originates in the roots. **Journal of Experimental Botany**, v. 38, p. 1174 –1181, 1987.

ZIMMERMANN, U.; BENTRUP, F. W.; HAASE, A. Xylem pressure, flow and sap composition of trees determined by means of the xylem pressure probe. In: Terazawa, M. (Ed.). **Xylem sap utilization**. Saporo: Hokaido University Press, p. 59-70, 1995.

_____________ et al. Xylem water transport: is the available evidence consistent with the cohesion theory? **Plant, Cell and Environment**, n. 17, p. 545-551, 1994.