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DEVELOPMENT AND USES OF PRESSURE PROBE: A BREAKTHROUGH IN THE STUDY OF WATER RELATIONS IN PLANTS

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Water plays a vital role in various structural, physical and metabolic processes in plants. The study of plant water relations drew the attention of plant research scientists by the start of this century. To quantify the plant water relations various measurable physical parameters were designed such as water potential along with its components, i.e. turgor and osmotic pressure. Different techniques/equipments were fabricated for the purpose, however, most of those were having some theoretical and technical limitations. Therefore, pressure probe was developed by Hüsken *et al.* (1978), that could measure cell turgor pressure directly in individual cells. Various other parameters could also be measured using the same equipment. This equipment can provide better understanding of plant mechanisms under control and stressed conditions. Therefore, it is urgently needed in broadening up the research horizon in Pakistan. It will also introduce us to new and challenging aspects of plant water relations in various crops, fruits, vegetables, halophytes etc.

INTRODUCTION

The importance of water relations had been realized since the start of this century and scientists had started describing various aspects (Thoday, 1918). In the beginning these were discussed and studied at whole plant and/or crop levels. Later, the studies were concentrated at tissue and organ levels. Water potential was considered to be a suitable parameter, that could represent the plant water relations properly. To measure this parameter various techniques have been designed. Among these the pressure chamber or bomb (Scholander *et al.*, 1965; Tyree and Hammel, 1972) was considered to be the most suitable. However, it is accompanied with some potential errors, such as that in its theory the cell wall solutes have been ignored (Tomos, 1988) which can influence the cell water relations directly. Another error (Murphy and Smith, 1989) is that it does not measure the water potential directly instead it measures the hydrostatic pressure in xylem. This argument has further been supported by the findings of Zimmerman *et al.* (Personal communication) who measured the negative hydrostatic pressure in the xylem, using the same equipment.

Since 50's, emphasis has also been laid on the importance of water relations of individual plant cells (Thoday, 1950; Kamiya and Tazawa, 1956). An excellent review by Dainty (1963) has set the foundations of the modern plant cell water relations. During early studies some techniques were designed in this context such as plasmometry, transcellular osmosis, perfusion techniques, shrinking and swelling technique (Tomos, 1988). These techniques were aimed at measuring the hydrostatic component of water potential that could never be measured directly before, rather, it was calculated by the difference of water potential and osmotic

pressure. Some more attempts were made on the same lines and the initial success was achieved in giant algal cells of *Velonia, Nitella, Chara* and *Acetabularia*. The manometers contained air bubbles and turgor was determined from the change in dimension of the bubbles when sealed into the cell. This approach had a number of shortcomings such as that the gas in bubble could dissolve in the cell and the cell volume could change due to the compressibility of the air.

Therefore, a new equipment was urgently and desperately required that could fulfil the need of time and could be with much less limitations. An equipment, pressure probe, was fabricated by Zimmerman *et al.* in 1969 which could measure turgor pressure directly but only in giant celled algae. Later, various refined alterations done also by the same group (Steudle and Zimmermann, 1974; Steudle *et al.*, 1975) improved the technique to the level that it could measure cell turgor pressure in higher plants as well (Hüsken *et al.*, 1978; Zimmermann *et al.*, 1980). This allowed not only turgor measurements but also the transient manipulation of turgor pressure to allow hydraulic conductivity (L_p), volumetric elastic modulus (ϵ) and reflection coefficient (σ) to be measured *in situ* under different physiological conditions (Zimmermann and Steudle, 1978; Tyerman and Steudle, 1982; Jones *et al.*, 1983). Some more parameters such as transpiration in algae (Ortega *et al.*, 1988), cell wall transpiration tension and osmotic pressure of cell wall solutes in higher plants (Arif, 1990) could also be measured. This equipment along with a micro-freezing point osmometer can facilitate the direct measurement of cell osmotic pressure (Shackel, 1987). The single cell water potential can also be calculated from the difference of its two components, cell turgor and cell osmotic pressure. Further, it has been extended and combined with other techniques to compare water relations at the cell and tissue levels and to get an insight into the mechanisms and limitation of water transport in higher plants (Steudle, 1987). Another modification of the equipment has made it capable of recording cell turgor pressure at different depths within a specific tissue (Pritchard *et al.*, 1989).

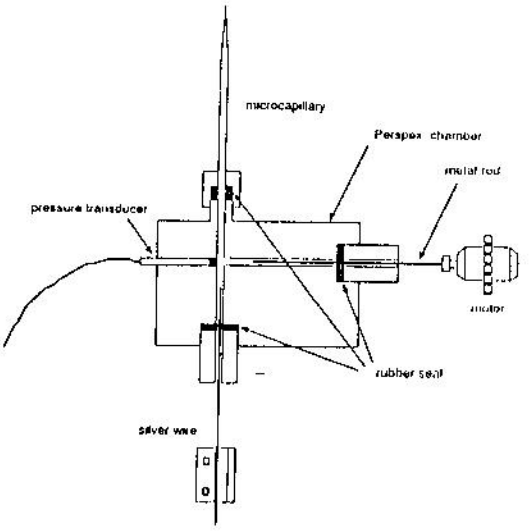

Fig. 1. The pressure probe.

The agriculture in Pakistan, being in semi-arid climate, is facing an acute problem of water shortage for crops that affects the plant water relations in particular and the other allied mechanisms in general.

Previously, the water relations have been studied in context of water deficit using pressure chamber technique (Tariq, 1987) though with the limitations discussed earlier. For better understanding of plant mechanisms under normal and stressed conditions, the research work needs to be extended to single cell resolution that will help in relating the new findings to the already existing knowledge. This can only be achieved if state-of-the-art technology be provided to the concerned research scientists in the country. Therefore, the pressure probe is urgently needed which will help in broadening up the research horizon in our country and will introduce to us many new and challenging aspects of plant water relations at cellular level.

Pressure probe: Pressure probe consists of a glass microcapillary, a solid state pressure transducer, a motor-driven piston and one manually operated piston. All these are connected to a Perspex chamber and the whole equipment is mounted on a micromanipulator (Fig. 1). The glass microcapillary is formed by extending 1 mm (i.d.) capillary to a tip diameter of 2-5 μm using a conventional electrical solenoid based puller. It is filled with low viscosity silicon oil and sealed into the oil-filled Perspex chamber with a rubber seal. The measurements are performed under a zoom stereomircoscope using a cool light from a fibre optic glass source to avoid the possible effect of warm light on the turgor pressure. The careful positioning of light source helps in finding out the sharp sap/oil meniscus and ultimately facilitates accurate measurements.

When tip of the microcapillary is inserted into the cell, the turgor pressure pushes back the oil into the microcapillary forming a meniscus at the oil/sap boundary (Fig. 2). Pressure in the chamber can be manipulated using a piston (either motor-driven or manually operated) until meniscus

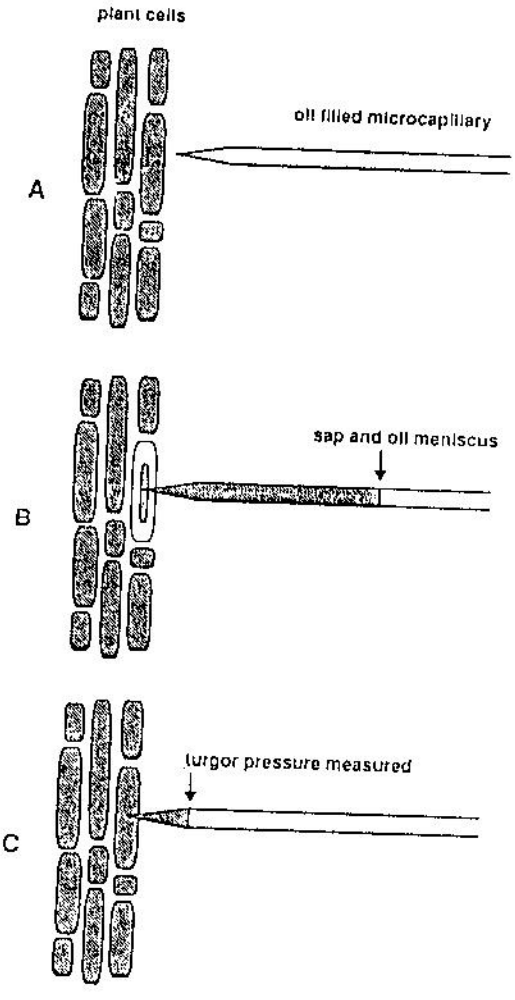

Fig. 2. The measurement of turgor pressure in single cell using pressure probe.

A. silicon oil filled microcapillary and the cell,

B. on insertion of microcapillary into the cell, turgor pressure pushes the sap/oil meniscus back,

C. pressure in the equipment is increased using piston (either motor-driven or mutually operated) to push the meniscus back and keep it stationary as near to cell wall as practicable.

is pushed back and is stationary as near to the cell wall as practicable. The pressure is transmitted by the silicon oil to the pressure transducer which converts it into a proportional voltage. Voltage output is then sent to a chart recorder via a control box which helps in an immediate visual control. Hence, a permanent record of the measurements can be obtained.

lowed to grow for 24 hours, then the outer sheath is removed to confirm the position of the growing zone by means of the zone of maximum displacement of the fine holes apart. The growing zone is about 5-10 mm away from the base of stem in wheat (Arif, 1990). The outer sheath is not removed completely to avoid the possible loss of turgor pressure by excessive evapotarnspiration

Table 1. Measurement of turgor pressure in single cells of wheat leaf and root

Cell type	Turgor pressure (MPa)	Reference
Leaf		
Mature epidermis	1.00	(Arif, 1990)
Mature mesophyll	0.98	(Arif, 1990)
Growing epidermis	0.55	(Arif, 1990)
Root		
Mature cortex	0.68	(Jones *et al.*, 1983)
Elongating cortex	0.65	(Pritchard *et al.*, 1987)
Mature epidermis	0.55	(Jones *et al.*, 1983)

Measurement technique: Various parameters can be measured using the pressure probe (discussed in previous section). However, in this review the method for the measurement of turgor pressure in epidermal cells (the first layer of cells in roots and leaves) will only be described, considering the space limitations. Moreover, this is the parameter the equipment was primarily designed for. For other parameters readers may consult the concerned literature as cited.

In leaf growing zone: The position of the growing zone in the leaf is determined by piercing fine holes through the stem of intact plant at regular intervals from the base i.e. root/shoot interface (Kemp, 1980; Thomas *et al.*, 1989). The plant is then allowed to grow for 24 hours, then the outer sheath is removed to confirm the position of the growing zone by means of the zone of maximum displacement of the fine holes apart. The growing zone is about 5-10 mm away from the base of stem in wheat (Arif, 1990). The outer sheath is not removed completely to avoid the possible loss of turgor pressure by excessive evapotarnspiration from the exposed surface. Instead, a window (2 x 2.5 mm) is cut carefully with a scalpel through the outer sheath at about 5 mm away from the stem base. In the majority of experiments the procedure is believed not to interfere with the growth rates (Thomas *et al.*, 1989). The growing zone is clamped horizontally to have a better view of the meniscus, in a Perspex leaf holder mounted on a micromanipulator.

In root growing zone: The root is held in position using a root Perspex chamber with screwed cover mounted on a micromanipulator. This provides a better control in manipulating the position of plant cells so that the measurements can be made with ease and accuracy. The root is kept immersed in distilled water or in the hydroponic medium,

usually covered with tissue paper soaked in the medium. The measurements are made in the region between 3-6 mm away from the root tip in case of wheat. This corresponds to a distance of 2.5-5.5 mm away from the base of the root cap since the root cap is 0.5 mm in length (Pritchard *et al.*, 1988; 1989).

In leaf and root mature zones: Both the tissues are also held in Perspex sample holder mounted on a micromanipulator. The measurements are performed in mature and fully expanded cells. In case of leaf the mature cells are generally about 5-8 cm away from the base of one weak old leaves (Arif, 1990)). However, root mature cells are located in between 6-20 mm away from root tip in one week old plants (Pritchard *et al.*, 1988). The leaf is kept in air and measurements are made on both surfaces, abaxial and adaxial, of it. However, the root is kept immersed either in distilled water or in the hydroponic medium during the measurements.

Turgor pressure has been measured in various plant types and in different tissues (Tomos, 1988). A brief account of its measurements in different types of wheat leaf and root cells has been given in Table 1.

Future prospects: In our country the equipment can be exploited efficiently and be used in studying various aspects of plant water relations and allied mechanisms, as already discussed. The studies can be carried out in single cells at different stages of plant development in control as well as in stressed plants, suffering from water, salt, temperature and nutritional stresses. Moreover, the studies can be extended to several types of crop plants such as wheat, cotton, rice, maize, sugarcane etc., and in various vegetables, halophytes, shrubs, weeds and fruits as well.

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