Freezing Processes in the Crown of 'Hudson' Barley, *Hordeum vulgare* (L., emend. Lam.) Hudson

Charles Robert Olien

**UNDER** normal conditions for growth, water is distributed between the space inside and outside of the living protoplasts in a balanced relationship (3, 5, 6, 8). The outer liquid, as contrasted to the inner liquid, forms a continuous system throughout the tissues where solutes can diffuse freely. The outer liquid is located along the cell walls, in spaces between cells, and in xylem vessels. Freezing causes redistribution of water with respect to both location and state (7). The pattern of redistribution of water during freezing determines the type and extent of stress (10). Some patterns of redistribution and, therefore, some types of freezing stress affecting various regions of plants can be distinguished by the electrophoretic method. This method is based on the principles of electrophoresis, on investigations of the path of migration of electrolytes through plant tissues in an electric field, and on studies of electrical properties of films over surfaces such as cellulose. Briefly, application of the method consists of measuring the direct current which passes between two electrical contacts on the plant at a series of low voltages. Data are taken continuously as the temperature is gradually lowered.

Studies of electrical properties and limitations of plant tissues and the migration path of indicators such as charged dye molecules showed that the weak direct electric current was conducted by migration of electrolytes through the continuous liquid system between living protoplasts (9, 10). The amount of liquid between protoplasts depends on the type of tissue and the moisture content of the plant. Usually most of the water is inside the protoplasts (in the protoplasm and vacuoles). Estimates of water in the free space have been as high as 23% for normal barley roots and 24.5 to 35.5% for normal wheat roots (2, 4). Corrections by Levitt indicate that the free space may be as low as 10% (8). In any case there is sufficient liquid to support electrophoresis of introduced substances along the cell walls in normal tissue (9, 10). Since mobility of these substances is greatly affected by plant moisture content it seems that liquid in the free space must also vary. Amaranth, a negatively charged dye, was used in many experiments to find the path of current through leaves, roots, and crowns of cereals and leaves of Canada thistle (9, 10). The path of migration was observed to be restricted to the liquid between protoplasts. Other substances such as methylene blue which are taken up by the protoplasts also were seen moving along the cell walls in the same manner as they do in a strip of moist filter paper through which a weak direct current is flowing. Electrophoretic migration of several other substances including silver, copper, 2,4-D, and a stem rust toxin have been studied (9, 10). Anderson et al. used this technique to study the mobility of calcium (1). While foreign substances introduced into

The tissues were observed to move along the cell walls, inclusions within protoplasts did not appear to be displaced by the weak voltages used in these studies. Also, no direct interference with metabolic activity was found in protoplasts under electrical treatment. Normal growth of crown tissues and normal development of rust lesions continued during treatments of several days.

The path of current was also evaluated from data on other electrical properties of plant tissues (9, 10). The organization of living tissue constitutes an effective barrier to the diffusion of internal electrolytes when a low voltage is applied. For example, the resistance of wheat leaves decreased to less than 5% of the original value after the leaves were killed provided the moisture content was kept constant. The barrier was reduced to a direct current when less than 5 volts per cm. were applied since within this range the resistance of the tissue was independent of the voltage used. When slightly higher voltages were used, the resistance decreased reversibly with increasing voltage indicating a weakening of the barrier. The diffusion barrier was irreversibly destroyed and the tissue killed when the voltage was greater than 20 volts per cm. The approach of injury during freezing can be evaluated by measuring the current at a series of low voltages since the maximum voltage which can be applied without affecting the resistance of the tissue decreases just above the killing temperature.

Experiments using various frequencies of alternating current also indicate that much of the electrolyte is restrained within protoplasts. The impedance decreases with increasing frequency since short vibrational displacements are not so greatly restrained. The effect of freezing on the total water content has been estimated by measuring conductivity using alternating current. In other experiments where the moisture content of tissues was rapidly readjusted, the resistance of tissue to direct current was found to be independent of the degree of cell contraction and directly dependent on the amount of liquid in the space between protoplasts.

**Types of Freezing Patterns**

The electrophoretic method of studying stress caused by freezing was used to recognize the temperature at which ice began to form and to identify the type of freezing process. Freezing processes were evaluated from data on the electrical properties of tissue which permit relating temperature with the relative content of liquid water between protoplasts according to the method described previously (10). A sample of the data, calculations, and a freezing pattern are presented in Table 1 and Figure 1. Three principal types of freezing patterns have been identified in barley and are illustrated in Figure 2.

When the temperature of hardy plant tissues is lowered freezing occurs in the space between protoplasts introducing a new phase and establishing a new relationship controlling the distribution of water. At constant temperature the vapor pressure in the remaining liquid becomes the same as that of the ice. The content of liquid water between protoplasts comes to equilibrium with both the content of ice and the content of liquid remaining in the protoplasts since the volumes shift until all three phases have the same vapor pressure. A slight decrease in temperature decreases the vapor pressure of ice more than that of the remaining liquid resulting in a shift in the distribution equilibrium.