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Ion Efflux in Cotton Roots at Different Stages of Development and Temperature Regimes

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Cotton seeds were germinated in incubator for 48 and 60 h. The root radicles were divided in several groups. In one group, the roots were separated into two equal halves ie. proximal and distal, in another group they were separated as long and short roots. These tissues were incubated at 35°C to analyse the electrical conductivity. In another experiment 48 and 60 h old root radicles were incubated at 30, 35 and 40°C to measure the electrical conductivity. Distal parts of roots released more electrolytes than proximal parts and short roots effluxed more electrolytes than the long roots. The 48 h old roots effluxed about double the electrolytes than 60h old roots.

Key words: Cotton roots, Electrical conductivity, Ion efflux.

Introduction

Maintenance of an adequate concentration of cations and anions plays an important role in osmoregulation in the cell. For example, Ca^{2+} helps in regulation of membrane permeability to various ions (Van Steveninck 1965). According to Wiles (1959) adequate quantity of Ca^{2+} is particularly important in young cotton seedlings for protection from the invasion of deleterious microbial organisms as a consequence of membrane or cell wall damage. Removal of Ca^{2+} from the plasma membrane also enhances leakage of intercellular K^+ (Weinberg *et al* 1983) which leads to the inhibition of root growth and elongation and metabolic processes (Hanson 1984).

When the environmental conditions are altered beyond normal limits under which a plant grows, the cellular membranes are found to undergo gross structural changes (Sullivan 1972). These changes may include phase separation of membrane constituents (Quinn 1988) and loss of its normal configuration and as a result the membranes become more permeable (Berry and Bjorkman 1980; Raison *et al* 1980). Not only electrolytes leak out through the damaged membranes, but larger molecules such as sugars, proteins etc are also released. However, the extent of this is much more dependent upon the degree of the membrane damage and nature of the membrane.

Quantification of membrane permeability and damage to cell membranes can provide valuable information about several aspects of plant performance including viability and vigour of seeds. Therefore present investigation was carried out to quantify the amount of electrolytes released from different parts of roots and from the roots of different ages at several temperatures.

Materials and Methods

The study was conducted at the School of Biological Sciences, University of Wales, Bangor (U.K.) in the year 1991-92. Acid delinted and sterilized seeds of cotton Cv. S-12 (obtained from Cotton Research Station Multan, Pakistan) were germinated at 25°C for 48 or 60 hours.

After 60 h of germination, the 30 longest roots were selected and divided into 2 groups. One group of 15 roots were cut into 1cm segments with a scalpel starting from their growing tips. In the second group of 15 roots, each root was cut into 2 equal halves: the proximal (root part near the cotyledons) and distal (root part away from cotyledons). Each root half was again cut into 1 cm segments. Thus, three lots of root segments were obtained, consisting of segments from "whole roots", "distal halves", and "proximal halves".

The second batch of 60 h old roots were divided according to their lengths. The first group contained the "long roots", while the second group contained the roots which were about half or less the lengths of long roots and are referred as "short roots". Fifteen roots were taken from each group and were cut into 1cm segments for the electrolyte efflux analysis upto 150 min at 35°C.

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The third batch of roots were grown for 48 or 60 h periods and were used without any subdivision. They were cut into 1 cm segments, however, and were subjected to incubation for 150 min at 30, 35 or 40°C.

Throughout the study the roots less than 3 mm length were excluded. The root segments were washed with distilled water and damp dried for about 5 seconds on filter paper. Immediately after drying, each lot was put into a 100 cm^3 conical flask containing 20 cm^3 sterile distilled water prewarmed at required incubation temperature (as mentioned above) in a shaking waterbath with shaking speed of 100 oscillation min^{-1}. The EC of the medium was measured using a conductivity meter (electrode size 1 cm) and the readings were taken at 10 min intervals for the first hour and 20 min interval during the second hour of incubation. A final reading was taken after further 30 min incubation. In all, the total incubation period was 150 minutes. The experiment was repeated 3 times. The curves were drawn on personal computer by SYSTAT program in Distance Weighted Least Squares Smoothing.

Results and Discussion

The data for electrolyte efflux from total roots and their halves are presented in Figure1. Electrolyte efflux from the whole roots and from proximal halves was almost same throughout 150 min incubation period. The distal halves of the roots gave a larger electrolyte efflux right from the start of the incubation upto the last reading (150 min). This volume is about 25 and 30% higher than the corresponding values for the proximal halves and whole roots, respectively.

The 150 min data for the three tissues in Figure 1 A were analyzed statistically using Student's T test. This analysis showed that the efflux value for the distal halves of the roots was significantly different at 5% level from the corresponding values for the whole roots and the proximal halves. There was no significant difference however, between the whole roots and the proximal halves.

For the purpose of comparison, the data for the "whole roots" electrolyte efflux is presented in Fig 1 A. In Fig 1 B they are referred as "mixed roots" against which short and long roots can be compared. It is apparent from Fig 1B that the electrolyte efflux from short roots was considerably greater than that from long roots. The corresponding EC values for the mixed roots were between the EC values for the short and long roots. The 150 min EC value for the long roots was about 35% below the value for the short roots and about 27% lower than the value for the mixed length roots. Application of the Student's T test to the150 min values showed a significant difference at 5% level between the value for the mixed

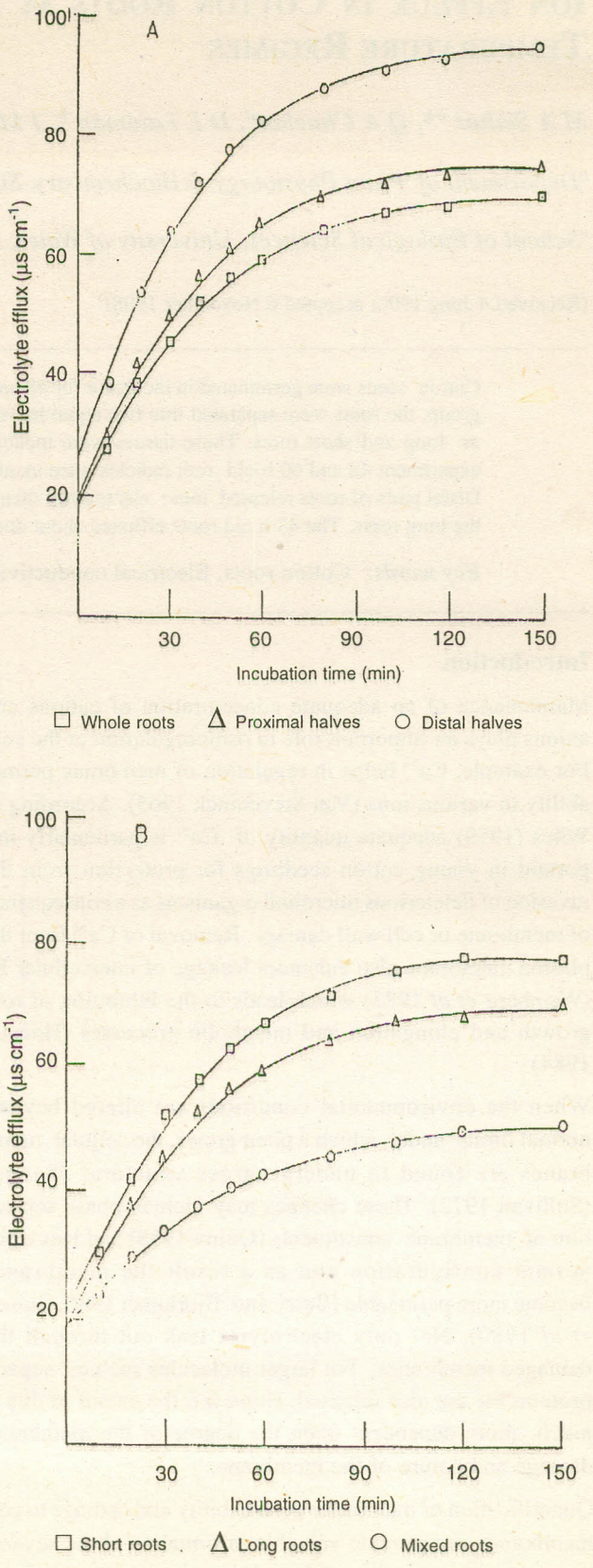

Fig 1. Electrolyte efflux from cotton roots.

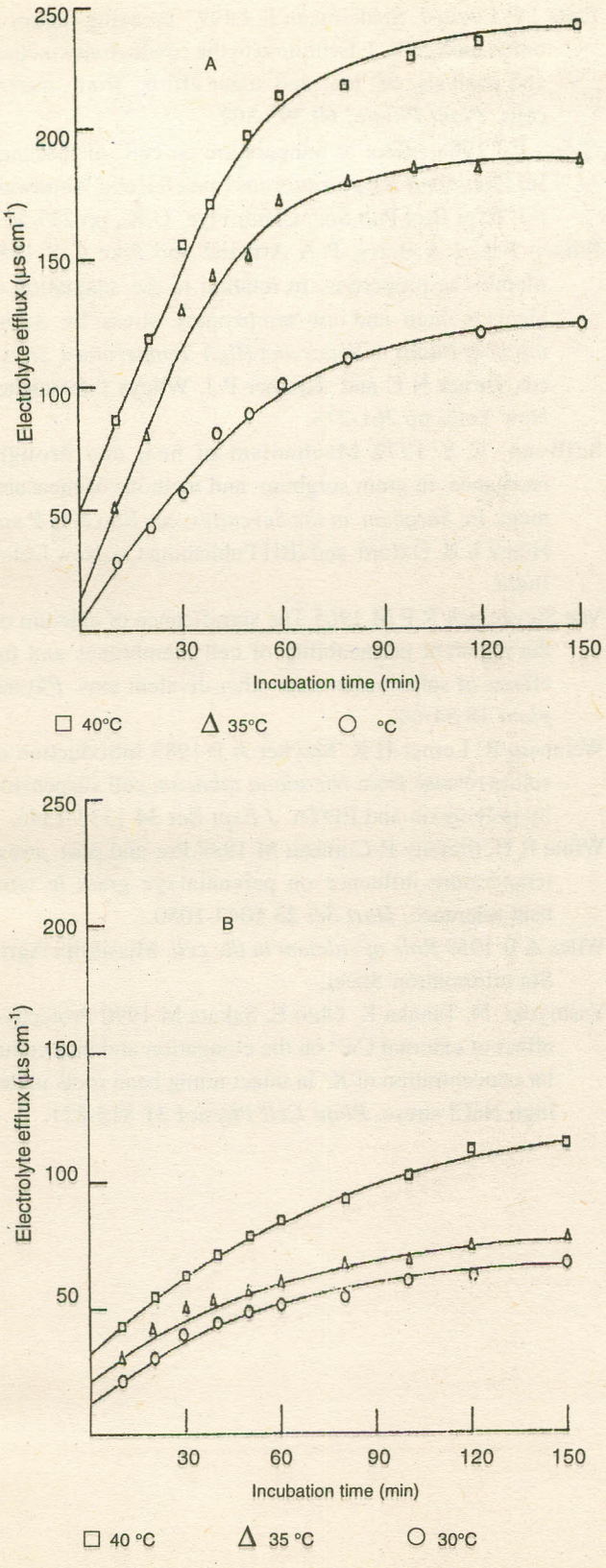

Fig 2. Electrolyte efflux from cotton roots (A: 48; B: 60 h old).

length roots and the value for the long roots. Similarly, there was a significant difference between the values for the long and short roots. There was no significant difference between the values for the mixed length roots and short roots, however.

The EC values of root radicals of 48 and 60 h old tissues are given as Figure 2. The data show that at all the temperatures the 48h old roots effluxed about double the electrolytes per gram tissue than those of 60h old roots. The 48h old roots effluxed 123, 187 and 240 µS cm^{-1} while 60h old roots recorded only 66, 76 and 113 µS cm^{-1} after 150 min period incubation at 30, 35 and 40°C respectively. This indicates that the 60h old roots had relatively more developed membrane structure than 48h old roots.

Measurements of the permeability of the cell membranes to water, ions and non-electrolytes is a sensitive experimental indicator of membrane damage (Palta and Li 1978). An important aspect of the solute loss following treatment at higher temperatures is the "time factor" or the kinetics of the leakage process. In this context White *et al* (1988) stated that the cumulative electrolyte leakage increased with time. Hallion (1975) and Palta *et al* (1977) further reported that electrolyte efflux eventually ceased during fairly prolonged incubation times. In the present study the rate of electrolyte efflux was progressively increased during the first 90 min of incubation and remained very slow upto 150 min incubation period which confirms our findings.

When the tissue is injured by any stress, membrane permeability increases and electrolytes diffuse out of the cell (Martineau *et al* 1979). This allows the assessment of relative damage by measuring the amount of electrolyte leakage. However, the effects of heat stress may not be equal in all parts of the plant (Koklacheva *et al* 1986). Palta *et al* (1977) also concluded that the leakage did not occur in all the cells equally, but weak and senescent cells were affected first by the stress. Dexter *et al* (1932) carried out experiments by exposing alfalfa roots to cold temperatures (5°C). They stated that the root parts which were buried more deeply in the soil (distal parts) suffered greater electrolyte loss than the root parts near the surface (proximal parts). It appears the tissue near the surface becomes hardened by exposures to the cold. They further stated that longer roots seemed to be distinctly more cold tolerant than the smaller roots as measured by the electrolyte leakage. The results reported in the present study lead to similar conclusion where the short roots leaked more electrolytes than long roots and distal halves leaked more than their proximal halves.

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