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CONDUCTIVITY STUDY OF IONS EFFLUX FROM SOAKED PEA SEEDS

Grażyna B. Plenzler, Leszek Cieśla

Department of Physics, University of Agriculture, ul. Wojska Polskiego 38/42, 60-637 Poznań
e-mail: gplenzler@woodcock.au.poznan.pl

Abstract. Measurement of the leakage of electrolytes after exposure to defined stress is a well-established, rapid and simple test for the quality and viability of seeds. The influence of the temperature of ion leakage, the preliminary imbibition of seed and the temperature of the seed in storage prior to soaking was examined in the experiments described. The amount of leaking ions rapidly increased with the drop in the temperature of the soaking medium, while the pre-soak storage temperature had no significant influence on the leaking of the ions. The results obtained can be explained assuming that the membranes are the primary targets of temperature treatment. A correlation between absorbance and the electroconductivity measurements was found. As concluded from the comparison of these measurements, protein components are not the only constituent of the leakage solution.

Keywords: pea seed, conductivity test, electrolyte leakage, membranes

INTRODUCTION

Old and low-quality seeds often leak more exudates than do high-quality seeds during the first hours of being soaked. Therefore the quality of seeds is assessed by a conductivity test based on measurements of the electroconductivity of the solutions obtained after soaking the seeds in deionised water. Over time, seeds leak a wide range of both organic and inorganic compounds such as sugars, amino acids, phosphorous and potassium ions, certain enzymes [8] and natural reserve compounds formed during seed development [12].

The electroconductivity of leakage solution from seeds is inversely correlated to emergence in the field under a variety of seedbed conditions [15]. It has been shown that the electroconductivity of the test solution changes after irradiation of the seeds with red and far-red light. The photoreversibility of such light treatment indicates that the effect observed is a phytochrome-mediated reaction [9].

It is well known that alterations in the properties of cellular membranes are involved in the regulation of different processes such as dormancy, chilling damage and secondary dormancy [1,3-5,7,11]. Some changes in the organisation of seed cell membranes occur in seed development and maturation, in post-dormancy seed storage and during imbibition before germination. Rehydrated seeds at the early phase of water uptake activate the ability of their cellular membranes to recognise and repair any damage that may have occurred in previous stages [6].

The electrolyte release mechanism was a hitherto unknown phenomenon. An explanation of this problem is very important and should provide information about subcellular structures and the components included in the ion efflux observed. The aim of the study is the designation of some essential features of the leakage process during the soaking of pea seeds and has been undertaken in an attempt to determine some of the components leaked from the seeds.

MATERIALS AND METHODS

The seeds (cv. Sześciotygodniowy) were specially selected in order to give approximately the same mass and volume. Twenty pea seeds in triplicate were exposed to preliminary imbibition on three layers of blotting paper in Petri dishes at 294 K for 20 hours. They were subsequently soaked in 50 ml of deionised water and incubated at a constant temperature: 282 K, 294 K and 308 K. Such estimated temperatures allow the activation energy of electrolyte leakage to be calculated on an 'Arrhenius' graph. In another part of the experiment, air-dried seeds were soaked in the conditions listed above.

Electroconductivity was measured by a microcomputer conductivity meter CC-317 and expressed in mS $cm^{-1} g^{-1}$. Absorbance of the test solution (per gram of seed mass) was observed at 250-350 nm and recorded simultaneously with the results of the electroconductivity measurements using a VSU-2P spectrometer.

RESULTS AND DISCUSSION

The kinetic curves for the leaching of electrolytes in seeds as recorded for pea seeds stored in deionised water at different temperatures are presented in figure 1. As can be seen, electroconductivity is strongly dependent on temperature and increases five-fold when the temperature increases from 282 K to 308 K. Those seeds soaked with deionised water at 308 K did not germinate having been placed on moist blotting paper at 294 K. Seeds soaked at 282 K and 294 K germinated completely under the experimental conditions employed.

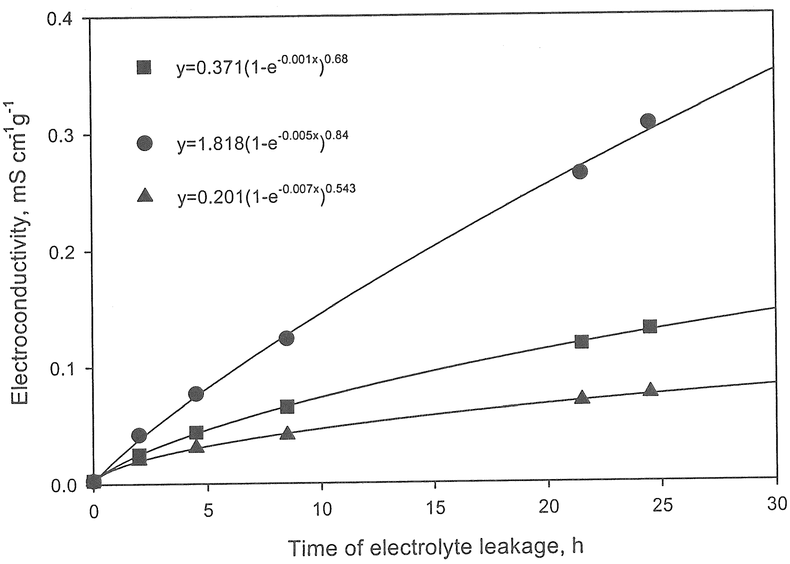

Fig. 1. The kinetic curves for the leaching of electrolyte in seeds as recorded for pea seeds stored in deionised water at different temperatures (●-308 K, ■-294 K, ▲-282 K)

It is generally accepted that the high electroconductivity of test solutions is an indicator of the low vigour and viability of seeds [5,6]. A decrease in vigour may be a result of high temperature treatment. In many works [2,5,7,10] membranes have been suggested as the primary target for the perception of temperature. The primary effect of temperature is the structural disturbance of the membrane. Changes in fluidity are accompanied by a number of secondary effects, such as a change in the permeability of the membrane or an alteration of protein conformation and function. The significant increase in the electroconductivity of the seed leachting solution at the highest temperature (308 K) may be attributed to lesions occurring in the plasma and the tonoplast membrane.

The dependence of temperature on the leakage of electrolyte from cell membranes is characterised by the Arrhenius activity energy value [13,14]. The value obtained is about 37 kJ mol^{-1}, which corresponds to the temperature coefficient Q_{10} of 1.48.

Figure 2 presents the influence of storage temperature on ion leakage from pea seeds. The electroconductivity of the test solutions measured after the immersion of air-dried seeds in deionised water is practically independent of the value of the temperature at which the seeds were stored before the soaking process. However, electroconductivity of the test solution for air-dried seeds is different from that recorded for seeds which had previously been imbibed (fig. 3). The differences are not significant and are best seen in the first phase of water sorption. The air-dried seeds soaked up the medium rapidly and thus the amount of out-flowing ions was higher as a result of the damage occasioned by the soaking process. The rapid soaking up of water disrupts membrane integrity and increases electrolyte leakage and in extreme cases may even block germination.

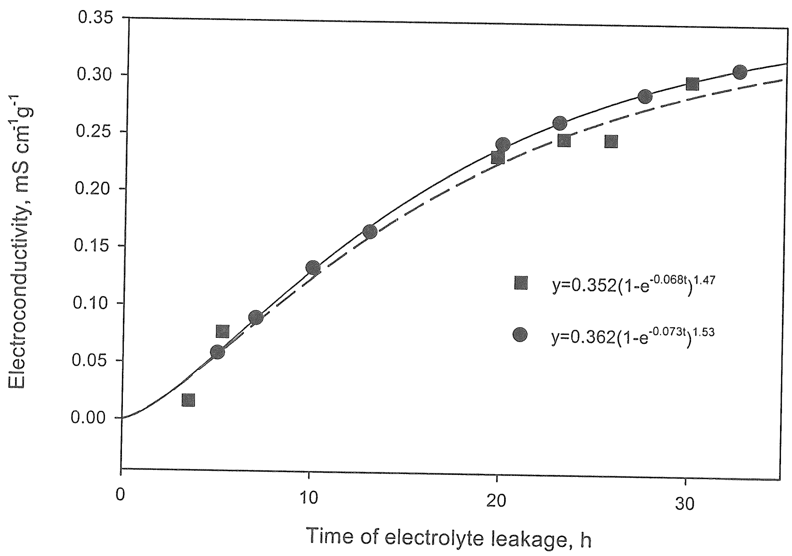

Fig. 2. The influence of storage temperature on ion leakage from pea seeds (•-air-dried seeds stored before the electrolyte leakage test at 294 K, ■-air-dried seeds stored at 282 K)

To determine which substance participated in the pea test solutions, the absorption spectra were recorded in 250-350 nm ranges. The absorption maximum at 265 nm indicates the presence of amino acids. As indicated by the data presented in figure 4, there is a correlation between the test solution electroconductivity and absorbance at 265 nm. As can be seen, neutral particles are also present in the solutions investigated, which have an appropriate value of absorbance and a zero electroconductivity value.

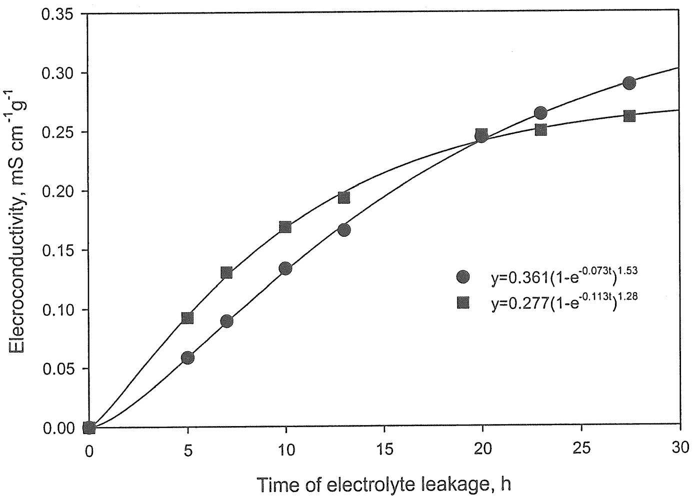

Fig. 3. The influence of preliminary soaking on the kinetics of leakage of ions from pea seeds (●-air-dried seeds placed in deionized water, ■-after preliminary imbibition)

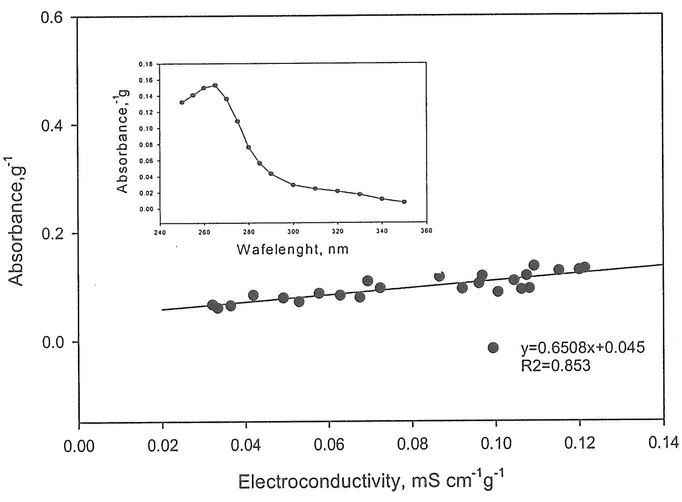

Fig. 4. Correlation between absorbance at 265 nm and the electroconductivity of the water solution of ions leaked from pea seeds. Insert shows the absorption spectrum in UV-range

The leakage of electrolytes from seeds has been examined as a result of imbibitional damage so far. We think that rather an analysis of two processes must be performed, namely: a damage during water uptake and the repair of this accumulated damage which allows seeds to germinate. Definite enzyme systems, from which the phytochrome cannot be excluded [4], may take part in the second process.

CONCLUSIONS

1. The process of ion release occurs during the soaking of seeds. The amount of leaking ions rapidly increases with growing temperature.

2. The results of the electroconductivity of the test solution obtained for different temperatures can be explained assuming that the membranes are the primary targets of temperature treatment.

3. The outflow of amino acids from the membranes has been indicated by the results of the measurements of absorbence. As may be concluded from comparison of the absorbence measurements and electroconductivity, protein components are not the only constituent of the solution to the question of leakage.

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BADANIA KONDUKTOMETRYCZNE UWALNIANIA JONÓW Z PĘCZNIEJĄCYCH NASION GROCHU

Grażyna B. Plenzler, Leszek Cieśla

Katedra Fizyki AR, ul. Wojska Polskiego 38/42, 60-637 Poznań
e-mail: gplenzler @woodcock.au.poznan.pl

Streszczenie. Pomiary uwalniania elektrolitów komórkowych po ekspozycji na określony stres są dobrze opracowanym, szybkim i prostym testem pozwalającym ocenić jakość i żywotność nasion. Zbadano wpływ temperatury, w której nasiona zanurzone w wodzie dejonizowanej uwalniają jony, wstępnego nawilżania nasion oraz temperatury przechowywania nasion na proces uwalniania jonów. Stwierdzono, że ich ilość wzrasta wraz ze wzrostem temperatury, w której ten proces zachodzi, natomiast nie zależy od temperatury przechowywania nasion przed kiełkowaniem Wykazano, że istnieje prosta korelacja pomiędzy absorbancją roztworów testowych oraz ich elektroprzewodnictwem i z analizy wzajemnej zależności pomiędzy tymi wielkościami wnioskowano, że składniki białkowe stanowią tylko część uwalnianych jonów.

Słowa kluczowe: nasiona grochu, test konduktometryczny, uwalnianie jonów, membrany