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SHORT COMMUNICATION

METABOLIC CHANGES IN NUCLEIC ACIDS ASSOCIATED WITH THE DEVELOPMENT OF SUCCULENCE

F. M. CATARINO* and A. J. TREWAVAS

Biological Sciences, University of East Anglia, Norwich, Norfolk

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Abstract—This report shows that the development of succulence in *Lobularia maritima* may be associated with a specific promotion of the labelling of transfer RNA.

INTRODUCTION

TREATMENT of the leaves of *Lobularia maritima* (Cruciferae) with a 2% solution of sodium chloride results in an increase of succulence through growth by cell-wall distension and inhibition of meristematic activity of the stem apex.[1] Associated with this increased succulence are, firstly, a promotion of the labelling of nucleic acids,[1] and secondly, a marked increase in endopolyploidy of leaf tissues. This communication reports an analysis of the former effect on nucleic acid metabolism using polyacrylamide gel electrophoresis.

RESULTS

The effects of sodium chloride on the labelling of the nucleic acids were first determined by separating the labelled nucleic acids on 2·5% acrylamide gels. After scanning at 265 nm the gels were sliced into 0·5-mm sections and counted. For analysis two treatment times with salt were chosen corresponding to time periods in which a general promotion of RNA synthesis by salt has been observed.[1] The radioactivity in each of three regions, high mol. wt. RNA, ribosomal RNA and soluble RNA were summated and the specific activities determined. The effect of salt on the specific activities of each of these three regions is shown in Table 1.

The results show that after 1 hr salt treatment there is an increase in the specific activity of the high mol. wt. RNA and ribosomal RNA of 29% but only 9% in soluble RNA. In contrast, 48 hr after salt treatment there is an increase of 40% in the labelling of the soluble RNA but only 20% in the labelling of the ribosomal RNA. Although in one experiment a large increase in the labelling of high mol. wt. RNA was observed, this effect was not reproducible on subsequent occasions. No specific differences were found between the four ribosomal components and consequently the salt effects on this fraction have been averaged.

The most consistent result in the 48 hr treatment was the promotion of labelling of soluble RNA. This fraction has been shown to contain at least four components in nucleic acids from green plants.[2] Briefly these are 4 and 5s from the chloroplast and 4 and 5s from the cytoplasm. To examine the effects of salt on the 4 and 5s RNA, labelled nucleic acids were

* Present address: Instituto Botanico, Faculdade de Ciencias, Lisboa, Portugal.

[1] C. C. CATARINO, *Endopoliploidia e Diferenciacao*, Doctorate Thesis, University of Lisbon (1968).
[2] T. A. DYER, *Phytochem.* 6, 456 (1967).

TABLE 1. THE EFFECT OF SALT TREATMENT ON THE LABELLING OF THE NUCLEIC ACIDS OF LEAVES OF *Lobularia*

Time after salt treatment (hr)	% Increase in specific activity due to salt treatment		
	High mol. wt. RNA	Ribosomal RNA	Soluble RNA
1	+29	+29	+9
48	+57	+15	+38
48	+4	+26	+46
48	+7	+18	+40

Single plants of *Lobularia* were sprayed either with 20 g/l. NaCl or with water. At the times indicated on the table, young leaves were harvested from both plants and incubated for 1 hr in 0·5 mc ^{3}H-adenine. The nucleic acids were isolated and separated on 2·5% polyacrylamide gels. After scanning at 265 nm the gels were cut into 0·5 mm sections and counted. The total counts in each of the three regions above were summated and the specific activities of each region determined. High mol. wt. RNA represents labelled nucleic acid running more slowly than ribosomal RNA.

electrophoresed for 3 hr on 7·5% acrylamide gels under which conditions complete separation of the 4 and 5s RNA was obtained. After scanning the gels were sliced and counted. Specific activities were determined and are shown in Table 2. Under the conditions used for electrophoresis ribosomal RNA penetrated about 2–3 mm into the gel and consequently specific activities for this fraction have also been included. The results show that there is a very distinct promotion of the labelling of the 4s RNA, compared to other fractions of nucleic acid, when plants are treated with salt.

TABLE 2. THE EFFECT OF SALT TREATMENT ON THE LABELLING OF THE LOW mol. wt. NUCLEIC ACIDS OF *Lobularia maritima*

Fraction	Specific activity (counts/min/μg)		% Increase
	Salt-treated	Control	
Ribosomal RNA	70·5	57	+24
5s RNA	24	17·8	+35
4s RNA	65	34·7	+87

Plants were treated either with a single application of water or 20 g/l. NaCl. After 48 hr young leaves were removed, incubated for 1 hr in ^{3}H-adenine and the nucleic acids extracted and separated on 7·5% acrylamide gels. After freezing the gels were sliced into 0·5-mm sections and the slices dissolved in 30% H_2O_2. After mixing with scintillator the dissolved slices were counted.

It could be supposed that the production of succulence is the result of specific alterations in the synthesis of proteins. Stent[3] has proposed a theory which explains how alterations in the synthesis of certain transfer RNA's may result in the enhanced synthesis of certain proteins. It will clearly be of interest to determine whether the development of succulence is an example of such a control.

[3] G. S. STENT, *Science* **144**, 816 (1964).

EXPERIMENTAL

Mature plants of *Lobularia maritima* were maintained under normal greenhouse conditions. Salt treatment was carried out by a single application of 20 g/l. NaCl solution using an aerosol spray.

Nucleic acids were labelled by incubating about 1 g fr. wt. of the younger leaves in 0·5 mc 2,8-^{3}H adenine (2 c/mM) for 1 hr. Nucleic acids were extracted by grinding the fresh tissue in a pestle and mortar with equal volumes of 6% *p*-aminosalicyclate and phenol mixture (phenol, 80%; *m*-cresol, 10%; 8-hydroxyquinoline, 0·01%; $MgCl_2$, 0·01%). The resulting mixture was stirred for 15 min; after centrifugation, two more phenol extractions were carried out on the clear supernatant. The nucleic acids were finally precipitated from the aqueous layer with 2 vol. of ethanol. Polysaccharide material was removed by the method of Ralph and Bellamy.[4] The nucleic acids were separated on 2½% and 7½% polyacrylamide gels as described by Loening,[5] except that the buffer contained, in addition, 0·2% sodium lauryl sulphate and 0·005 M $MgCl_2$. Separations were carried out at 5 mA/gel for 2–3 hr at room temp. After scanning at 265 nm, the gels were frozen and sliced into 0·5 mm sections. The sections were dissolved in 1 ml 30% H_2O_2 by gentle heating and the resulting solution mixed with 15 ml dioxan: cellosolve (5:1) containing 1% PPO, 0·05% dimethyl POPOP and naphthalene 5%.[6] All samples were counted in a Packard scintillation counter.

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[4] R. K. Ralph and A. R. Bellamy, *Biochim. Biophys. Acta* **87**, 9 (1964).
[5] U. E. Loening, *Biochem. J.* **102**, 251 (1967).
[6] G. A. Bruno and J. E. Christian, *Anal. Chem.* **33**, 1216 (1961).