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STUDIES OF INDUCED THERMOSENSITIVITY IN WHEAT ROOTS

by

INGRID SKOGQVIST

UPPSALA 1974

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Doctoral thesis at Uppsala University 1974
ISBN 91-554-0186-4

Printed in Sweden by
Uppsala Offset Center AB 1974

PREFACE

This dissertation is a summary and a conclusion of aspects of heat treatment on wheat roots. The investigations were performed at the Institute of Physiological Botany of the University of Uppsala, Uppsala, Sweden. Results obtained have been presented in the following separate papers:

I. Skogqvist, I. & Fries, N. 1970. Induction of thermosensitivity and salt sensitivity in wheat roots (Triticum aestivum) and the effect of kinetin. - Experientia 26:1160-1162.

II. Skogqvist, I. 1973. Induction of thermosensitivity in wheat roots: salt sensitivity and effects of chloramphenicol and ethanol. - Physiol. Plant. 28:77-80.

III. Skogqvist, I. 1974. Effect of chloramphenicol, ethanol, kinetin and oligomycin on salt sensitivity and adenosine triphosphate content of wheat roots. - Z. Pflanzenphysiol. 72:297-304.

IV. Skogqvist, I. 1974. Induction of heat sensitivity of wheat roots and its effects on mitochondria, adenosine triphosphate, triglyceride and total lipid content. - Exptl. Cell Res. 86, June No. (In press).

V. Skogqvist, I. 1974. Heat sensitivity of wheat roots and protecting effect of ethanol and kinetin. -

VI. Skogqvist, I. 1974. Some metabolic inhibitors influencing the response of wheat roots to high temperature treatments. -

The fore-mentioned papers will be referred to by Roman numerals.

INTRODUCTION

The temperature range over which plants can survive in nature is about 90°C. However, the difference in the minimum and maximum temperatures where most plants are able to grow is about 30°C. Growth of wheat can occur between low temperatures of -2°C to 5°C and high temperatures of 30°C to 37°C, whereas more thermophilic species such as corn and cotton have temperature maxima as high as 45°C. Friend et al. (1962, 1965) studied the effect of temperature on the growth of wheat. They found the highest relative growth rate at 30°C, but this growth rate declined with increasing plant age and the decline accelerated at temperatures above 30°C.

Plant roots in general have a lower temperature optimum for growth than shoots. Roots are more sensitive to sudden temperature fluctuations (Muromtsev 1962).

Resistance to heat and cold is thought to be closely associated with the colloidal and chemical properties of the protoplasm (Langridge and McWilliam 1967). These include the degree of protoplasmic viscosity and the phase changes in the membrane lipids (Singer and Nicolson 1972, Raison 1973). Heat-resistant plants have been shown to possess protective mechanisms based on respiration. A respiration decrease caused by heat results in an accumulation of organic acids, which function as a substrate for detoxification of endogenous ammonia produced at high temperature (Petinov and Molotkovskii 1960). Protecting effects of sugars against injury caused by high temperatures were reported by Molotkovskii and Zhestkova (1964). They suggested that sugars block the active surface of mitochondria membranes. Sakai (1965) showed that sugars also are important in cold resistance; the greater the cold resistance the higher the rate of conversion of starch into sugar.

A heat shock can produce either an increase in heat tolerance or an increase in heat sensitivity, depending on the experimental conditions. Yarwood (1961) showed that a treatment for 30 seconds at 50°C is sufficient to increase the heat tolerance of some cultivated plants. Fries and Söderström (1963) reported that many organisms can be made sensitive to supraoptimal temperatures if they are heat-shocked and immediately thereafter incubated at the supraoptimal temperature. In the present paper the author's investigations on the induction of thermosensitivity and salt sensitivity in wheat roots by means of heat shock or chemical treatments are summarized from the original papers I-VI. Compounds which have a protecting effect against heat and salt treatment are briefly presented and their effects are discussed. The influence of heat shock on cell structure in the wheat root meristem is also reviewed. For further details the original papers should be consulted.

RESULTS AND DISCUSSION

The influence of heat shock on wheat root meristem cells.

The most suitable temperature for the heat shock of wheat proved to be 45°C for 2-minute treatments (I) and it was found that roots are more sensitive to this temperature than shoots. The root meristem died if placed at a supraoptimal incubation temperature after the heat treatment. Thus it is not a question of heat-hardening but of a heat-sensitizing effect.

After a heat shock UV-absorbing material, with an absorption maximum at 260 nm, leaks into the surrounding medium (VI). Glucose also leaks through the cell membrane but only at 12°C after the heat treatment. Those roots, which have a glucose leakage before being placed at 35°C, grow very well at 35°C, even better than unheated roots (I).

It has been shown that the sugar content of corn root cells increases at lower temperatures (Patterson et al. 1972). If it is the same with wheat root cells such an increased glucose content may be reduced at 12°C by leakage to the surrounding medium. This means that less respiratory substrate is available in the cell, i.e. the metabolic rate is lowered also when the heat-treated plants are transferred to 35°C.

In IV the main interest is focused on the heat shock itself. Immediately after the heat treatment the content of free triglycerides increased in the root tips, but the content of total lipids did not change. Thus a synthesis of triglycerides seems to take place at the expense of other lipids already available in the cell. It could be that these other lipids are derived from the cell membranes, notably from phospholipids and/or galactosylglycerides, because the permeability is changed by the heat treatment (VI, cfr. also Mazliak´s (1973) scheme).

In electron microscope studies (IV) no immediate effect of the heat shock at 25°C could be observed. When incubated at 35°C the meristem cells of the heat-shocked root tip were plasmolysed, soon became very swollen, and subsequently died. After 24 hours new centers of meristem cells appeared just behind the dead apical meristem. From these centers lateral roots were produced. These roots grew very well and gave a high fresh and dry weight. This was the reason why fresh and dry weight were avoided as measures of the sensitizing effect induced by heat.

As shown by Altergot and Dzhekshenaliev (1973) thermal injury mobilizes metabolites from one part of the plant for regeneration of cells and organs in another part of the plant. They heat-treated the upper part of a leaf and studied the regeneration of roots.

In the present study (IV) heat-treated roots incubated at 25°C for 45 minutes after shock gave nearly normal mitochondria in the meristem cells whereas the endoplasmic reticulum cisternae widened and formed vacuoles. The Golgi vesicles became irregular. About 4 hours incubation at 25°C after heat treatment gave a normal appearance of endoplasmic reticulum and Gogli vesicles in the meristem cells. On the other hand, the mitochondria at the same time had become irregular and had few tubules. Close to the mitochondria membrane curls containing lipids could be observed. They were absent after 24 hours, while the meristem cells were restored to their former condition.

The meristem cells of the heat-treated roots incubated at 35°C died and most of the cell content was lysed (IV). Langvad (1972) observed the same in a fungus, Merulius lacrymans, at supraoptimal temperature. He found that the mitochondria were very heat sensitive, their structures becoming profoundly decomposed. Other authors (see Langridge and McWilliams 1967) also showed that the mitochondria are sensitive to heat and claimed that the thermal damage is the result of an uncoupling of oxidative phosphorylation. However, determinations of the ATP content in heat-treated wheat root tips showed that the ATP values vary from 30 to 140 % of those from the control roots during the first 50 minutes after heat shock and thereafter the values stabilize at about control levels (IV).

Some metabolic inhibitors influencing the response of wheat roots to high temperature treatment.

In VI it is shown that 10^{-5} M 2,4-dinitrophenol (DNP) produces a sensitivity to supraoptimal temperature, if the wheat roots are placed in DNP solution and then transferred to high temperature. This compound also has a protecting effect against the consequences of the heat shock at the same concentration, if the root tips are treated in DNP solution before heat treatment and incubation at supraoptimal temperature. DNP is a true uncoupler, the respiration of the cell may continue normally, but no coupled phosphorylation of ADP to ATP takes place.

Oligomycin, monofluoroacetate and nitrogen have the same protecting effect as DNP, if the roots are treated with these compounds before heat shock. Oligomycin influences some of the membrane-bound ATP-ases. Monofluoroacetate inhibits aconitase in the tricarboxylic acid cycle. Nitrogen atmosphere decreases the respiration of roots.

Chloramphenicol (II) also shows a protecting effect against the deleterious influence of the heat shock. This compound is known to suppress oxidative phosphorylation, because it interferes with 50S - 70S ribosomes and thus inhibits the synthesis of cytochrome proteins in the mitochondria.

All these above-mentioned compounds influence the mitochondria and their function in the cell. It seems that if the rate of metabolism in the mitochondria decreases before heat treatment all root meristems survive at supraoptimal temperature. If the roots were treated with these compounds after the heat shock very often all root meristems died.

Puromycin (VI) has also been examined and does not show any protecting effect against the influence of the heat treatment. A pretreatment with puromycin did not protect against the effect of the heat shock, but caused an increased sensitivity to heat. Puromycin is believed to act at the level of ribosomal protein synthesis.

The effect of ethanol and kinetin on wheat roots.

Ethanol and kinetin also protect the root meristems against the deleterious consequences of the heat treatment (I, II, V). Both compounds may influence the membrane permeability of the cell. One hour in ethanol solution (3×10^{-2} M) before heat treatment is enough to protect the root meristems against heat shock, but about 15 hours in kinetin solution (10^{-6} M) are required for the same effect. These two compounds obviously do not act by reducing the amount of free triglycerides, which are formed under heat treatment, becuase the content of triglycerides increases (IV, V).

A possible explanation of the kinetin effect is that this compound prevents the formation or activation of hydrolytic enzymes such as proteinase, peptidase, RNase and β-1,3-glucan hydrolase, which are often membrane-bound. In this way the cell may survive because it is protected against hydrolytic breakdown.

Both ethanol and kinetin decrease the ATP content of root tips (III). However, their alteration of ATP content probably is not related to their influence on heat sensitivity, because chloramphenicol, which also protects the root meristems against heat shock, increases the ATP content.

Induced salt sensitivity in wheat roots.

Heat treatment of wheat root meristems not only sensitizes them to supraoptimal temperature but also to high salt concentrations (I, II). Ammonium, potassium and sodium salt of chloride, bromide and iodide have been tested and the heat-treated root tips proved to be much more sensitive than untreated root tips.

Fries (1972) found that the amino acid, histidine, and other imidazole compounds protect the fungus, Ophiostoma, against induced salt sensitivity. With wheat root tips histidine has no effect on heat and salt sensitivity (unpublished).

Some of the compounds already found to protect against the deleterious influence of the heat shock were tested for a protective effect against a high salt concentration (III). Chloramphenicol, ethanol, kinetin and oligomycin were examined and all these compounds were found to protect the roots against salt treatment. There does not seem to be any obvious correlation between the capacity of these compounds to protect against salt exposure and their effect upon the ATP level.

A membrane theory applied to the effect of heat treatment.

Singer and Nicolson (1972) presented a fluid mosaic model of biological membranes. There the fluid mosaic structure is formally analogous to a two-dimensionally oriented solution of integral proteins (or lipoproteins) in the viscous phospholipid bilayer solvent. The proteins are a heterogeneous set of globular molecules, which are arranged with the ionic and highly polar groups towards and into the aqueous phase and the nonpolar groups in the hydrophobic interior of the membranes. These globular proteins are partially embedded in a matrix of phospholipids, which are organized as a discontinuous fluid bilayer. A small fraction of the lipids may interact specifically with the membrane proteins.

In mitochondria from chilling-sensitive plants and animals it has been found that the activity rate of membrane-associated enzymes is suddenly changed at a certain low temperature (Raison 1973). This change appears at about 15°C and may depend on a phase change in membrane lipids (the lipids probably are in a solid state below this temperature). In chilling-resistant plants and animals no such change can be observed down to about 0°C, probably because more unsaturated lipids are formed at low temperatures (Kleinschmidt and McMahon 1970).

Chapman and Urbina (1971) determined the phase transition of lipids found in membranes of Mycoplasma laidlawii to occur at temperatures between 10°C and ca. 25°C for the neutral lipids fraction, between 15°C and ca. 40°C for the glycolipids and phospholipids fractions, and between ca. 20°C and ca. 40°C for total lipids of intact membranes.

In the present investigation the heat shock temperature is above the transition temperatures in the fore-mentioned studies. One can therefore expect the heat shock to cause a disorganisation of the membrane lipid pattern and therefore probably also a displacement and structural alteration of the globular membrane proteins. The membrane curls (IV) may visually represent the membrane disorganisation induced by the heat treatment.

ACKNOWLEDGEMENTS

I am deeply indebted to Professor Nils Fries, Head of the Institute of Physiological Botany, University of Uppsala. He introduced me to this theme of research and his never failing interest, constant encouragement and support have been of great importance to me.

For valuable discussion, helpful criticism and assistance with ATP determinations I wish to thank Professor Göran Stenlid, Department of Physiological Botany, Agricultural College, Uppsala.

I am also indebted to Professor Karl-Heinz Kiessling and Dr. Erik Fellenius, the Institute of Zoophysiology, Uppsala, for valuable discussion and helpful criticism about the ethanol effect and help with the determination of triglyceride content of wheat roots.

I also thank Docent Angelica v. Hofsten for introducing me to the techniques of electron microscopy.

Many thanks are also due to Mrs. Pia Isaksson, Miss Ingvor Andersson, Miss Anette Eriksson, Mr. Rein Calm, Miss Margaretha Melin, Mr. Åke Danielsson and Miss Barbro Johansson for excellent technical assistance, and to Miss Inga-Britt Jansson for typing the manuscripts.

I am grateful to Dr. Howard Bonnett, Dr. Sagar Krupa, Dr. Edgar DaSilva and Dr. Linda Fryklund for revising the English text.

I also take this opportunity to convey my thanks to my dear husband Mr. Leif Skogqvist who has been a fountain of encouragement, inspiration and support.

Financial support has been provided by P.O. Lundells Fond and Olof Wiks Minne.

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Editor: The Dean of the Faculty of Science

Doctoral dissertations from the Faculty of Science are published either as monographs or as summaries of articles previously printed. It is these summaries that are collected in the series "Abstracts of Uppsala Dissertations from the Faculty of Science".

Distributed by
Almqvist & Wiksell
Gamla Brogatan 26
STOCKHOLM, SWEDEN

Uppsala Offset Center AB 1974

ISBN 91-554-0186-4