TRANSPORT OF SUBSTRATE WITHIN THE WHEAT GRAIN

by

TRELAWNEY DAVID UGALDE

Department of Plant Physiology,
Waite Agricultural Research Institute,
University of Adelaide,
South Australia.

Thesis submitted for degree of
Doctor of Philosophy

1987
TABLE OF CONTENTS

SUMMARY

STATEMENT

ACKNOWLEDGEMENTS

ABBREVIATIONS

SECTION 1, GENERAL INTRODUCTION

1.1 Wheat production in Australia and the aims of this review

1.2 Grain quality

1.2.1 Texture of the endosperm

1.2.2 Physical and chemical attributes of grain carbohydrate

1.2.3 Attributes of grain protein

1.2.3.1 Protein quality

1.2.3.1.1 Soluble proteins

1.2.3.1.2 Gluten proteins

1.2.3.1.3 Structure, role and heritability of glutenin proteins

1.2.3.1.4 Structure, role and heritability of gliadin proteins

1.2.3.1.5 Environmental influence on protein quality

1.2.3.2 Protein quantity

1.2.3.2.1 Role of protein quantity in determining end-product use

1.2.3.2.2 Breeding for increased quantity

1.2.3.2.3 Environmental influence on grain protein percentage

1.2.3.3 Nutritional quality of wheat protein

1.3 Quality and yield of irrigated wheat
1.3.1 Factors limiting carbohydrate deposition in the grain  &  21
1.3.2 Factors limiting protein deposition in the grain  &  24
1.3.3 Strategy for high yields and high protein percentage  &  25
1.4 Grain development  &  26
  1.4.1 The flower and early development of the ovary and ovule  &  27
  1.4.2 The vascular system of the grain  &  29
  1.4.3 Anatomy of tissues surrounding the grain during the period of grain fill  &  30
    1.4.3.1 Outer pericarp  &  30
    1.4.3.2 Inner pericarp  &  30
    1.4.3.3 Seed coat and pigment strand  &  31
    1.4.3.4 Nucellar epidermis and nucellar projection  &  32
  1.4.4 Development of the endosperm  &  32
    1.4.4.1 Cellular development  &  32
    1.4.4.2 Accumulation of the storage product  &  36
1.5 Biochemical pathways of starch synthesis  &  38
1.6 Biochemical pathways of protein synthesis  &  38
1.7 Ontogenetic and distributional patterns of starch and protein deposition in the endosperm  &  40
1.8 Transport of substrate into and within the grain  &  43
  1.8.1 Route of substrate movement  &  43
  1.8.2 Role of intercellular invertase  &  49
1.9 Carbon fixation by the inner pericarp and transport of the photosynthetic product to the endosperm  &  49
1.10 Concluding remarks and outline of research project  &  51

SECTION 2, MATERIALS AND METHODS
2.1 Source of materials
   2.1.1 Wheat plants
   2.1.2 Soil mix
   2.1.3 Chemicals and reagents
   2.1.4 Water

2.2 Growing plants
   2.2.1 Environment
   2.2.2 Plant culture
   2.2.3 Using anthesis as the reference date

2.3 Manipulative treatments
   2.3.1 Trimming the ear
   2.3.2 Degraining spikelets
   2.3.2 Disrupting putative transport pathways
       2.3.3.1 Disrupting transport in the stalk
       2.3.3.2 Disrupting transport in the vascular bundle
       2.3.3.3 Disrupting lateral transport in any circumferential pathway
   2.3.3.4 Control grains
   2.3.3.5 Prepared grains

2.4 Feeding radioactive substrate to wheat grains
   2.4.1 $^{14}$CO$_2$ to illuminated ears
   2.4.2 $^{3}$H-glutamine and $^{14}$C-sucrose to cultured spikelets

2.5 Sectioning the grain
   2.5.1 Dissecting the grain into component tissues
   2.5.2 Sectioning radially from the inner pericarp to the vascular bundle

2.6 Measuring dry weight
   2.6.1 Dry weight of whole grains
2.6.2 Dry weight of small sections of tissue

2.7 Measuring the volume of sections taken from the dorsal region of the grain

2.8 Measuring the volume of the endosperm cavity and the area of the endosperm cavity-endosperm interface

2.8.1 Measuring the volume by planimetry

2.8.2 Measuring the volume by expressing and weighing the sap

2.8.3 Measuring the area of the endosperm cavity-endosperm interface

2.9 Collecting endosperm cavity liquid for component analysis

2.10 Extracting solutes from tissues of the grain and floret

2.11 Separation of neutral and charged fractions in aqueous and ethanolic extracts

2.12 Measuring radioactivity

2.12.1 Ethanolic extracts

2.12.2 Aqueous extracts

2.12.3 Ethanol-insoluble material or whole tissue

2.12.4 Photosynthetic tissues of the spikelet

2.13 Measuring plant components by HPLC

2.13.1 Soluble carbohydrates

2.13.2 Free amino acids

2.13.3 Component amino acids of protein

2.14 Isolating starch

2.15 Isolating protein

2.16 Hydrolysing oligosaccharides for component analysis

SECTION 3, RESULTS
3.1 Physical characteristics of the endosperm cavity in six varieties of wheat and the effect of reducing the number of grains per ear

3.2 Solutes of the developing wheat grain: Using HPLC to measure the principle free amino acids and soluble carbohydrates

3.3 Association between substrate gradients within developing wheat endosperm and regional patterns of dry matter deposition

3.4 Route of $^{14}$C-carbohydrate movement within the wheat grain

3.5 Evidence for two transport mechanisms within the endosperm of wheat

3.6 Culture of detached wheat spikelets and the route of movement amino acids within the grain

SECTION 4, GENERAL DISCUSSION

BIBLIOGRAPHY
SUMMARY

In wheat, both yield and grain quality are determined to a large extent by factors intrinsic to the grain. The work reported in this thesis studies the transport of substrate within the grain and examines whether or not factors associated with this transport may influence yield and quality.

The first part of the experimental programme (sections 3.1, 3.2 and 3.3) used microsectioning, HPLC, and radiotracer techniques to (1) identify the solutes that form the principal substrate for starch and protein deposition in the grain, (2) describe the concentration gradients of these solutes throughout the endosperm and (3) test whether or not the regional patterns of deposition of the polymeric products within the endosperm could be due to the patterns of substrate supply.

Carbohydrate entered the grain as sucrose and most of the carbohydrate transported through the endosperm was transported as sucrose, even though sucrose accounted for only 40-50% (w/w) the total soluble carbohydrates. On the other hand, a wide range of amino acids formed the complement of amino-substrate. Sucrose, and indeed soluble carbohydrate as a whole, and amino acids did not conform to the same distribution pattern throughout the endosperm. But these differences in the distribution of substrate did not account entirely for the regional differences in the deposition of starch and protein. Also there were regional differences in the kinetics of conversion.

Overall, the rate of turnover of sucrose within the endosperm was nearly 10 times that of amino acids.

The second part (sections 3.4, 3.5 and 3.6) described the route of solute movement throughout the grain and tested whether or not the observed concentration patterns of substrate within the endosperm could be due to differences in the direction of substrate supply. Both carbohydrates and amino acids destined for the endosperm moved by the same route; i.e., longitudinally in the vascular tissue at the base of the crease, then
radially through the endosperm cavity and endosperm. There was no detectable movement of solutes into the endosperm in an inward radial direction from the inner pericarp or any other tissue surrounding the endosperm. Differences in the distribution of carbohydrate-substrate and of amino-substrate throughout the endosperm were due to kinetic factors within the endosperm itself.

The results are discussed in relation to the distribution of dry matter within the endosperm and to the source-sink interactions for carbohydrates and for nitrogenous compounds between the plant and grain as a whole.