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The influence of altered water availability on stomatal patterns of leaves.

by

Naziha Abdulkader El-Hashani

MSc., Garyounis University (Benghazi-Libya)

A thesis submitted for the degree of Doctor of Philosophy

Department of Biological Sciences
University of Durham
England (UK)

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This thesis is entirely my own work. It has not previously been submitted for any other degree or diploma.
The Mass Spectrometry analyses described in this work were performed at the University College of Wales, Aberystwyth.

Naziha Abadulkader El-Hashani

DEDICATED TO MY MOTHER
MY CHILDREN, BROTHERS AND SISTER

ABSTRACT

The aim of this research was to study the influence of **altered water availability on stomatal patterns of leaves.** A baseline study of leaves stomatal indices, (stomatal as a proportional of all epidermal cells) stomatal density, (number of stomatal per unit area of epidermis) epidermal cell density, epidermal cell length and stomatal length and widith was made in wheat, and sorghum cultivars; Saudi Red, Funk, Indian White, Morgon 854, Morgon 856 and Pioneer 8ss in relation to water limitation.

Seedlings of wheat and sorghum cultivars grown under water limitation showed increases in their stomatal density, epidermal cell density and trichome index in response to reduced watering frequency. Stomatal index of the two species, however, were not altered in the same way. In sorghum there was no apparent change in stomatal index in relation to water limitation treatment but stomatal density and trichome index increased.

In wheat there was a marked reduction in the stomatal index in response to water limitation but there were increases in trichome indices and stomatal densities of all parts of the leaves.

Wheat plants grown under water limitation imposed by polyethylene glycol (PEG) treatments to roots showed marked reductions in their growth compared with controls. Stomatal density was found to increase in response to PEG treatments while stomatal index decreased.

Incubation of newly-germinated wheat seedlings in PEG resulted in an inhibition of growth of coleoptiles. However, the density of stomata decreased, unlike what was seen in leaf tissues.

When wheat coleoptiles were subjected to conditions of limited water availability guard mother cell (GMC) production appeared not to be influenced by water status of the tissues.

Wheat coleoptiles grown under different periods of light showed slight decreases in stomata density compared to the controls.

Abscisic acid treatment was able to substitute for water limitation treatments by reducing stomatal index in both leaves and coleoptiles whilst increasing the production of trichomes in leaves. However, in coleoptile the same treatment reduced both the GMC index as well as stomata index.

Salinity treatments, inhibited seed germination and were found to decrease stomatal index.

Incubation of wheat seedlings in PEG resulted in a yellow pigmentation of the roots. The pigment was tentatively identified. However, it was not possible to distinguish whether the pigment was a specific metabolite of PEG in the roots or was produced by a non-biological conversion of PEG.

ABBREVIATIONS

ANOVA	=	Analysis of variance.
cm	=	Centimeter.
Ca (NO₃)₂. 4H₂O	=	Calcium nitrate.
CuSO₄	=	Copper sulphate.
df	=	Degrees of freedom.
ECD	=	Epidermal cells density.
EDTA	=	Ethylenediaminetetra-acetic acid (disodium salt).
F	=	Variance.
g	=	Gram.
h	=	Hour.
H₃BO₃	=	Boric acid.
KH₂PO₄	=	Potassium dihydrogen phosphate.
KCl	=	Potassium chloride.
K₂SO₄	=	Potassium sulphate.
l	=	Litre.
LR	=	London Resin Company Ltd.
mg	=	Milligram.
ml	=	Millilitre.
mm²	=	Square millimeter.
mM	=	Millimolar.
μ	=	Micron.
MgSO₄.7H₂O	=	Magnesium sulphate.
MnSO₄	=	Manganese sulphate.
nd	=	Not determined.
NG	=	No growth.
ns	=	Non significant.
NS	=	Evans and Nason's nutrient solution.
Na₂MO₄	=	Sodium molybdate.
PEG	=	Polyethlene glycol.
P	=	Probability.
ppm	=	Parts per million.
PBS	=	Phosphate buffer saline.
SI	=	Stomatal index.
SD	=	Stomatal density.
UV	=	Ultra violet.
ZnSO₄	=	Zinc sulphate.
w/v	=	Weight for volume.
SE	=	stander error.
no	=	number.

ACKNOWLEDGMENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1
INTRODUCTION

1.1 Water stress

Water is the matrix of the life, contributing as much to the properties of life as such complex molecules as carbohydrates, fats, and proteins. Water is directly or indirectly required for every life process and every chemical reaction; it is a vital component of most of these reactions, the medium in which they all take place, and the solvent for most of the reacting chemicals.

Water is the continuous phase between the cells, and within the cell and is an essential part of every plant tissue, of tissues, and of the cell protoplasm. Depending on the tissue and type of plant, approximately 80 to 90 percent of the plant is water. It is an integral component of the proteins and nucleic acids which are essential to the cell. Water is vital to these macromolecules since its properties of hydration allow reactivity between molecules in solution and between enzymes and substrates. The rate and physiological activity of many metabolic reactions depend on the degree of hydration of enzymes and other reactants.

Water is vital to every stage of plant development from seed germination to plant maturation. Germination proceeds only after seeds have imbibed sufficient water for rehydration and cell growth. As water diffuses into the seed, the protoplast becomes rehydrated, food reserves are hydrolized, and

the enzymes begin to function. Starch is digested to sugars, lipids to soluble materials and storage proteins to amino acids. Energy is then liberated in respiration, foods are translocated, and embryo growth begins. Once growth is initiated, the water supply must be sustained to provide for cell expansion, and the hydrogen needed for carbohydrate production for incorporation into the protoplasm of the new cells.

Water is further required to provide the constant pressure of turgor which supports the plant and facilitates cell enlargement after the new cells have been initiated. As the cells mature, water accumulates in the vacuole, building up a pressure which forces the protoplast against the cell wall, keeping it fully distended or turgid. The turgidity of the cells, tissues, and the entire plant depends entirely on an ample water content. Water also provides the medium in which essential mineral nutrients are carried.

Few people would dispute the conclusion that water availability is one of the two or three major factors determining the distribution of plants throughout the world. Lack of water has been a major selective force in plant evolution and ability to cope with water deficits is an important determinant of natural distribution of plants (Fischer and Turner1978). Accordingly, an understanding of the mechanisms that confer adaptation to dry environments holds much theoretical and practical value. Plant adaptations to such environments can be expressed at four levels: developmental, morphological, physiological and metabolic (Hanson 1980, Hansen and Hitz1982). Of these

levels, metabolic or biochemical adaptations to dry environments are the least known about and understood.

Plants often show a reduction or cessation of growth as one of the earliest discernible effects of a deficit of water. There is, however, considerable controversy about the relative sensitivity to water shortage of the two immediate components of growth, cell division and cell extension (Slatyer 1967; Kramer 1969). Usually, plants which show small reductions in growth in dry regimes are considered to be adapted to such conditions in nature. Leaf areas are generally less in the plant grown under dry regimes and this reduction may contribute to reductions in growth as well as any direct effects of water deficits upon photosynthesis.

The rate of growth of plant cells and the efficiency of physiological processes are highest in cells which are at maximum turgor. However, the absorption of carbon dioxide through moist cell walls exposed to the atmosphere, which is essential for photosynthesis, is associated with the loss of water from leaf tissues. Plant cells which have lost water, and are at a turgor pressure lower than the maximum value, are said to be suffering from water stress. Terry *et al* (1971) recorded a reduction in the rate of cell division, but no effect on cell expansion in leaves of sugar beet with extremely small reductions in the soil water potential. Hopkinson (1968) found that both primordium production and leaf expansion of tobacco were reduced by a water

deficit, the leaf expansion showing accelerated rate after recovery whereas primordium production did not.

Photosynthetic carbon assimilation is the paramount factor in determining plant productivity (Boyer 1982). There is much information on the effect of water stress on photosynthetic, CO_2 fixation and related processes (Hsiao 1973; Kriedmann and Downton 1981; Boyer 1982). Damesin and Rambal (1995), who worked with *Quercus pubescens*, reported that in this species water deficit generally induces stomatal closure and a simultaneous decrease in photosynthesis, however, the sensitivity and the response rate are species specific (Acherar and Rambal 1992).

In higher plants, water deficit often causes stomatal closure and a consequent reduction in photosynthesis, but photosynthesis is also reduced by water deficits in lower plants which have no stomata. This indicates a more complex action of water deficit on plant metabolism probably related to cell turgor and membrane to cell wall contact. Respiration may be affected only slightly by small changes in water content but larger reductions may cause increased respiration before an ultimate decline (Brix 1962). Meidner and Mansfield (1968) suggested that the combination of decreased photosynthesis and increased respiration in plants subjected to water deficits results in increased carbon dioxide concentrations in leaf mesophyll, which give the sensitivity of the stomatal apparatus to carbon dioxide, and may hasten stomatal closure.

Water stress also has adverse affects upon other processes such as cell division which appears to be less severely affected than cell expansion (Gardner and Niemann 1964). Growth may be further inhibited by changes in carbohydrate metabolism (Eaton and Ergle 1948), nitrogen metabolism (Shah and Loomis 1965) and possibly in the production of growth substances (Larson 1964), and disruption of the translocation of materials (Roberts 1964). The fact that many of these processes are controlled by enzymes suggests that water stress may affect proteins directly.

The drought resistance of a plant varies with its previous history and stage of growth as well as with conditions prevailing at the time of measurement. Plants in different physiological states may also vary in their drought resistance; chlorotic plants show little resistance to deficits (Hutchinson 1970) and a reduced ability to recover from this state. Shaded dwarf shrubs are also less able to resist the formation of deficits and more intolerant of low water contents than plants from the open (Bannister 1971). Most higher plants are exposed to varying degrees of water-stress at some stage in their development. The degree of water stress may be varied by small fluctuations in atmospheric humidity and net radiation in more mesic habitats to extreme soil water deficits and low humidity in arid environments. With the exception of the plants which tolerate extreme cellular dehydration, adaptation to water stress involves the reduction of cell dehydration by either avoidance or tolerance of stress (Gaff 1980). Some examples of avoidance of water stress

are rapid completion of development, leaf abscission, leaf rolling and low stomatal conductance to water vapour (Turner 1979). Tolerance to water-stress usually involves the development of low osmotic potentials which characterise many plant species found in more arid environments (Iijin 1957; Maxwell and Redmann1978; Walter1965).

It appears that a plant's reaction to increased water stress is complex but that most of the physiological changes are brought about by the changes in the water relations of the tissues of the plant itself. Stomatal movement, cell turgor and direct metabolic effects all influence the growth and hence the ecological response of the plant. Numerous reports and reviews on plant responses to drought have now been published (e.g., Bewley 1979; Bewley and Krochko 1982; Bradford and Hsiao 1982; Kramer 1983; Levitt 1980; Morgan 1984; Mussell and Staples 1979; Tranquillini 1982).

Total free amino acids in leaves are often increased if rather severe water stress lasts several days (Barnett and Naylar1966). Amides frequently increase but proline continues to be the most studied molecule under water stress or associated salinity stress in plants (Aspinall and Paleg 1981; Dix 1986; Ebenhardt and Wegmann 1989; Chowdhury *et al* 1993; Shashi *et al* 1995). The increase in proline was reported first by Kemble and Macpherson (1954). Stewart and Lee (1974), Chandler and Thorpe (1987), Le-Rudulier *et al* (1984), McCue and Hanson (1990), and Serrano and Gaxiola (1994) suggested that proline may serve as a storage compound for nitrogen, as an osmo-solute

and hydrophobic protectant for enzymes and cellular structures during stress. These properties are related to the low molecular weight and neutral status of proline.

A considerable number of investigations have studied the adaptive responses of crop plants to drought stress in order to identify traits that will allow more drought-resistant genotypes to be developed. Specific genes have been identified, such as those which are responsive to abscisic acid, the *rab* genes (Skriver and Mundy 1990),which may be involved in drought stress resistance. It may be possible to determine the value of these genes to a droughted plant by introducing enhanced or reduced expression of the gene into a crop species by genetic engineering techniques and studying the consequences. With the development of molecular markers for many crops, it is now possible to produce closely spaced genetic maps using an appropriate mapping population of plants. However, as yet no single gene for drought stress resistance has been identified. This may be because so many genes are involved in the response.

1.1.1 Experimental induction of water deficits in plants
1.1.1.1 Polyethylene glycol (PEG)

Because of difficulties in maintaining satisfactory control of soil moisture, the validity of some the field studies of plant water relations is questionable. When plants are growing in soil, studies of the effects of water

stress on physiology are usually either very short so that soil and plant water stress change little, or they may involve repeated soil drying cycles (Kaufmann 1968). For many studies, however, water stress of soil-grown plants cannot be manipulated well enough for careful experiments. Therefore, the addition of osmotic agents, such as (PEG), salts or sugars to liquid nutrient media is very useful and has received considerable attention in the literature (Janes 1966; Lawlor 1970; Slatyer 1961; Venkateswarlu *et al* 1993; Uma *et al* 1995; Jingxian and Kirkham 1995)

The use of osmotic solutes to provide controlled water potential around the roots offers opportunities for overcoming many uncertainties in field studies. The use of PEG to adjust osmotic potential requires an accurate knowledge of the effect of PEG on osmotic potentials. PEG can be used to decrease water potential of plants only if it does not enter the root and has no toxic effects (Lawlor 1970).

In many experiments on water relations, it is desirable to relate results to natural environmental situations. To do this when PEG or any other osmotic agent is used, it is important that water relations of the plants exposed to the osmotic agent are similar to those of plants in soil at the same water potential.

PEG has the general formula $HOCH_2 (OCH_2CH_2)_nOH$ being composed of neutral polymers available in a range of molecular weights which are, highly soluble in water and with low toxicity to mammals. Because of these properties they have been used by several investigators to imposed water stress on plants

by decreasing the water potential of the rooting medium and so the water potential of the plant (Lawlor 1970). PEG has other properties, however, which makes it less suitable for use as an osmoticum, these include lowering the surface tension and increasing the viscosity of the solution.

An earlier report, (Michel 1970) indicated that elongation of cucumber hypocotyl sections in growth regulator solution was suppressed more by Carbowax 6000 (PEG) than by Mannitol at equivalent water potentials .

In one of the earliest studies in which Carbowax was applied to plants Withrow and Howlett (1946) found that undiluted Carbowax 1500 and Carbowax 4000 were very toxic to tomato leaf and flower tissues. In fact they reported that tomato floral parts were injured by as little as 0.1% Carbowax in aqueous solution. But they noted that up to 1% Carbowax applied as a spray was non-toxic to leaf tissue of several plants. Raymond and Gingrich (1966) found that the wheat roots under Carbowax 6000 were yellow with the intensity of the yellow colour related to the amount of stress. PEG compounds of various molecular weights are used extensively for the experimental control of water stress in plants growing in nutrient solution. It is frequently assumed that plant water relations are similar whether the plants are growing in soil or in a PEG solution having an equal water potential. A main characteristic of PEG which has justified its use as an osmoticum is its assumed non-metabolism by plant tissues.

PEG 20,000 has been used to decrease the water potential of kidney bean (Lagerwerff *et al* 1961). Jarvis and Jarvis (1965) applied PEG 1540 to tree seedlings and Leshem (1966) grew pine seedlings in PEG 400 and 1500. Janes (1966) reported that bean and pepper grew satisfactory in PEG 600 solution. Use was made of PEG in this study in order to bring about changes in water deficit in plants. However, consideration was also given to potential adverse and metabolic effects of this compound.

1.1.1.2 Salinity

This can function at two levels; as an osmotic agent or as a toxic compound. Large terrestial areas of the world are affected by salinity. On a world-wide basis it is estimated that there are between 400 and 950 x 10^6 hectares of salt-affected soils (Epstein *et al* 1980). Soil salinity is one of the principal factors responsible for deterioration of soils, with consequent reduction of their agricultural potential. Saline-sodic soils in semi-arid regions pose difficulties in land use. The accumulation of soluble salts in soil leads to an increase in osmotic potential of the soil solution which may limit the absorption of water by the seeds or by the plant roots (Bal and Chattopadhyay 1985).

A general suppression of the growth is probably the most common plant response to salinity. Na^+ and Cl^- are among the more common ions found in excess in saline soils, and some plant species are specifically sensitive to one or both of these ions (Bernstein and Hayward1958). Salinity reduced the rate

of leaf elongation of non-halophytes. In barley, these reductions are rapid and may be related to a reduction in turgor (Thiel and Lauchli1988). Several hours after the start of salinity stress, however, leaf elongation is poorly correlated with turgor. Termaat *et al* (1985), and Munns and Termaat (1986) postulated that a non-hydraulic signal from the root limits the growth of the shoot when the plant is salt- stressed. One of the prime candidates for this putative signal is ABA. Munns *et al* (1995) who worked with wheat and barley reported that most changes in metabolism or gene expression leading to growth reductions under salt-stress related to the osmotic effect of salinity, not to any salt-specific effect.

Alternatively it was suggested that nutrition of the shoot apical meristem may be disturbed in the initial stages of salt stress and that shoot meristem might be the source of a signal to expanding leaves (Lazof and Lauchli 1991).

Crop plants differ greatly in their tolerance of salinity. Spinach and the garden beet are highly tolerant and often show a stimulation of growth by levels of NaCl that would be lethal for the pea (Hayward and Bernstein1958). An understanding of the physiological and biochemical attributes which enable a plant to survive under saline environment is a prerequisite for improving its cultivation on such soils. Plants growing on saline soils show considerable differences in physiological and biochemical activities, which result from adaptive mechanisms during evolution. Such adaptations could be deep-seated

at the molecular level, as in halophytic bacteria (Peterkin and Fitt 1971), or at the semimolecular level, as indicated by the formation of sodium-induced chloride-bound proteins which regulate metabolic activities of the plant cells (Strogonov *et al* 1973). These adaptations also cause readjustments in the activities of certain key enzymes of the plant metabolism, (Ahmad 1972; Ahmad *et al* 1974).

Effects of salt stress on germination and seedling growth have been studied by many workers in desert plants such as *Hammada elegans* (Mohmoud 1985). Kent and Lauchli (1958) conducted similar studies for Sunflower, Rice and Cotton. Salt effects on germination and seedling emergence of several vegetable crops (carrot, chile pepper and tomato) have been investigated amongst others by Miyamoto *et al* (1985).

1.1.1.3 Abscisic Acid

The stress responses of plants cannot be understood without some consideration of a role for Abscisic acid (ABA). ABA can act as a second messenger at the molecular, cellular and whole plant levels, where it is thought to translate environmental effects into biological responses.

Many authors have now drawn attention to the fact that substantial numbers of drought effects on plants can be mimicked by external application of ABA (e.g Davies *et al.* 1986). Most notably ABA application reduces water

loss by restricting leaf conductance and leaf area development but can also increase water uptake into roots. Observations of this type, coupled with the early observation that unwatered plants can show substantially enhanced ABA contents, have led to the suggestion that ABA may act as a "stress hormone" in droughted plants. The increase in ABA in dehydrated plant tissues was first detected by Wright and Hiron (1969) in excised bean leaves. Pierce and Raschke (1980) showed that when excied leaves of different species were dehydrated, ABA synthesis and metabolism were not affected until bulk leaf turgor was reduced to zero. Zhang and Davies (1990) have shown that the concentrations of ABA in the xylem of maize plants increase with increasing drought; growth and stomatal conductance are inhibited as the ABA concentrations rise. Thus, it would seem that roots may be the primary sensor for drought stress. In contrast to ideas that drought stress is related to a biochemical signal from the roots, Kramer (1988) argues that direct hydraulic effects are more important during shoot water stress.

It is important to note that stomatal reactions are often observed well before deficits of this magnitude develop in leaves and indeed are sometimes observed before any perturbation in shoot water relations can be detected. It is known that a reduction in turgor (Raschke 1982) or cell volume (Ackerson and Radin 1983) act as a trigger to increase ABA biosynthesis but we do not know where in the pathway this trigger acts.

The primary site of action of ABA in fully differentiated leaves is the outer surface of the guard cells (Hartung 1983; Hornberg and Weiler 1984). Abscisic acid appears to induce a net loss of potassium from the guard cell, with consequent reduction in turgor, cell shrinkage and closure of the stomatal pore. Stomatal closure requires much less ABA than is accumulated in water stressed leaves (Raschke 1982). In fact, ABA already present in turgid leaves at the onest of stress may be redistributed to give a rise in apoplastic ABA which results in stomatal closure (Zeevaart and Creelman 1988). Likewise, ABA originating in the roots can move to the leaves in the transpiration stream and affect stomatal aperture. Nevertheless there is evidence that some of these effects are also seen in other cells. Thus ABA-induced membrane depolarizations have been observed in a number of higher plant systems, for example, by Ballarin-Denti and Cocucci (1979) in radish seedlings, and by Kasamo (1981) in both epidermal and mesophyll cells from tobacco leaves.

ABA-induced increases in cytoplasmic Ca^{2+} have been observed in cells of corn root, corn coleoptiles and parsley hypocotyls by Gehring *et al* (1990), who suggest that this is likely to be a general phenomenon. Interestingly, the increase in cytoplasmic Ca^{2+} following ABA treatment is associated with an increase in cytoplasmic pH, whereas auxin produces an increase in cytoplasmic Ca^{2+} but a decrease in cytoplasmic pH. Gehring *et al* (1990) suggest that guard cells show the same pattern of changes. It is

therefore possible that changes in cytoplasmic pH also play a role in the transducation of the ABA signal into ionic changes in guard cells. Since these early findings there has been considerable interest in the relationship between ABA and the functioning of the stomatal apparatus, particularly with changing moisture status, and the subject has been reviewed by Raschke (1975). However, it is possible that ABA may have developmental effects in relation to water loss control in a longer time context.

1.2 Stomata

1.2.1 Occurrence and structure

An important aspect of the ability of plants to withstand water deficit conditions is the functionality of stomata. These control most of the water loss from the aerial parts of higher plants.

When plants first began to colonize land, only those which had evolved a hydrophobic waxy outer layer or cuticle were able to avoid desiccation (Martin *et al* 1983). The ability of the plant to lose water by evaporation to the aerial environment would be impeded by the presence of such a layer. Without the development of stomata in the epidermis the supply of carbon dioxide would have been insufficient to maintain land plant life. In higher plants, stomata control gas exchange, they allow carbon dioxide and oxygen in the air to diffuse in to the intercellular air spaces of the photosynthetic tissues. At the same time, however, there is a potential loss of water vapour via transpiration.

By controlling these two fundamental processes, both of which contribute to the productive efficiency of plants, stomatal movements play an important role in life of plants. True stomatal pores are a feature of land plants, it is convenient to apply the term stomata to the entire unit, the pore and the two guard cells although this is probably more conveniently refered to as the stomatal complex.

Discussions of stomatal structure often emphasize the existence of two types of stomata, with guard cells shaped either like a kidney or a dumbell (Allaway and Milthorpe 1976; Esau 1977; Palevitz 1981; Sack 1987; Louguet *et al* 1990). Kidney-shaped guard cells are found in most plants including mosses, ferns, gymnosperms, dicots, and many monocots. These stomata (paired guard cells) are roughly elliptical in outline in paradermal view, although there are many variations in this basic form.

Dumbell or bone-shaped guard cells are characteristic of grasses, and thus these stomata are also referred to as the "grass type". The nucleus in grass guard cells is also dumbell-shaped. In addition, the walls separating the two grass guard cells have relatively large openings that create areas of cytoplasmic continuity. All three features; dumbell-shaped guard cells and nuclei, and cytoplasmic continuity, have been described in all grass genera that have been examined (Flint and Moreland 1946; Brown and Johnson 1962; Kaufman *et al.* 1970; Srivastava and Sing 1972; Galatis 1980).

The stomatal complex may be surrounded by cells that do not differ from other ground cells of the epidermis, where they are obviously neighbouring cells. Often, however, subsidiary cells are distinguishable from other epidermal cells by microscopic observation. One feature that distinguishes subsidiary cells from other epidermal cells is that they are suspended above the sub-stomatal cavity. Subsidiary cells if present, are also included as part of the stomatal apparatus or the stomatal complex.

1.2.2 Distribution

In photosynthesizing leaves, stomata may occur on both sides or only on one side. About two-thirds of species have stomata in the lower epidermis only (abaxial surface) which are called hypostomatous leaves (Slavik 1974). The rest of species tend to be amphistomatous, with stomata on both epidermes. In these cases, the numbers of stomata in the lower epidermes of leaves tend to be greater than those in the upper epidermes, except in some of the Poaceae. The aquatic species with floating leaves, bearing stomata on the adaxial epidermis which are exposed to the atmosphere, are called epistomatous leaves. Submerged leaves of aquatic species have no stomata.

1.2.3 The functional response of stomata to environmental factors

Stomatal guard cell movements, in common with many other activities in plants, are greatly affected by the environment. Since stomata are the main sites of gaseous diffusion in leaves, it is not unexpected that the two factors which affect their behaviour on a daily bases are light and carbon dioxide which are integral in photosynthesis. However, other factors also play important parts either directly or indirectly e.g hydrology. In addition to function it also been shown that there are differences in stomata density of leaves of the same species, depending on the environment in which the leaves developed (Eckerson 1908; Rea 1921; Penfound 1931).

1.2.3.1 Light

Light is probably more important in determining the stomatal behavior than any other component of the environment. Most plants open their stomata during the day and close them during the night, the exception being CAM plants. Stomata have their own endogenous controls which express themselves in a circadian rhythm of opening and closing. For this reason stomata illuminated during the night hours are usually reluctant to open, and darkness during day may not produce complete closure.

The maximum speed of stomata movement differ from species to species. In some species the complete opening may take many hours, whereas

in other species the movement takes a few minutes and may be complete in half an hour, as for example in *Xanthium pennsylvanicum*. There is usually a saturation irradiance giving maximum stomatal movements but not in all plant groups (Burrows and Milthorpe 1976).

Florescent lights in the laboratory are often sufficient to induce stomata opening but their spectra are usually different from sunlight so there is a need for supplementation with incandescent lamps to be closer to the sunlight spectrum (Weyers and Meidner 1990). Another interesting aspect of stomatal behaviour is that adaxial guard cells are exposed to sunlight, the abaxial guard cells only receive reflected light. The quality of light reaching the abaxial surface of the leaf is largely filtered by the mesophyll and contains less than 10% of the blue and red light falling on the adaxial surfaces.

1.2.3.2 Carbon dioxide

Carbon dioxide makes a substantial contribution to the daily cycle of opening and closing of stomatal pores. Direct effects of carbon dioxide concentration on stomata were reviewed by Morison (1987). Different species show different responses, some are insensitive e.g., *Gossypium hisutum*, while others decrease in conductance as the carbon dioxide increases from 300 to 500 μ mol mol^{-}1 e.g., *Pyrus malus*. Maximum stomatal conductances are between 0 and 100 μ mol mol^{-1} carbon dioxide and the greatest effects on

stomatal pore are usually below 300 μ mol mol^{-1}. Scarth, in as early as 1932, suggested that the light- dark responses of stomata might be due to carbon dioxide influx through respiratory activity and carbon dioxide removal during photosynthetic activity. Within the physiological range of carbon dioxide levels it can be generally stated that increase in carbon dioxide concentration caused stomatal closure, and carbon dioxide level decreases caused opening of stomata.

1.2.3.3 Temperature

As early as 1898, Darwin noted that an increase in temperature resulted in an increased stomatal opening both in the light and in the dark but that it also slowed down the rate of the closing phase. Other reports show that stomatal opening may be either favoured or stopped by temperature increases. Loftfield (1921) further showed that an increase in temperature led to a decrease in the time taken for the stomata to reach their steady state opening phase. These various temperature effects may be as result of the interaction of temperature with carbon dioxide concentration. A good example of the way temperature affect stomata, movements through carbon dioxide concentration is to be found in the so-called "midday closure". In the plants growing in hot climates the stomata are completely open in the morning. A build up of carbon dioxide through a depression of photosynthetic efficiency at high temperature combined

with enhanced carbon dioxide production from photorespiration is also believed to be the cause of midday closure in certain species. This was substantiated by (Meidner and Heath 1959) who were able to reverse midday closure in onion at 34°C by blowing carbon dioxide free air through the intercellular spaces of the leaf.

1.2.3.4 Water

Plant water deficits occur when the rate of water loss from the plant exceeds the rate of water uptake from the soil. This is a situation which occurs daily, as a result excessive water loss from leaves in relation to the ability of the roots to replace the lost water More severe water deficits arise from a excessive shortage of soil water, either as result of drying or freezing. Larger deficits will also occur if transpiration rates are very high, for example, when the air is hot or dry. Leaf conductance is, however, ultimately determined by turgor levels in the epidermis (Kaufmann 1976; Lawlor and Lake 1976; Edwards and Meidner 1978) and may often be only loosely related to the water potential of the mesophyll cells. Stomatal density strongly influences water use efficiency in plants (Woodward 1987; Woodward and Bazzaz 1988; Mansfield *et al* 1990). Responses of stomata to water stress must be regarded as fundamental both to plant survival and efficient crop production.

The guard cells act as the first line of control of water stress by being able to regulate their aperture to prevent unnecessary transpiration losses at the same time as maintaining photosynthetic efficiency. In maize, the stomata close under severe drought of three or four days duration but recover in two days when then water supply is renewed. If the period of drought is longer, such as a week or more the after-effects on the stomata are permanent and they open to less than half their normal aperture, yet the leaves completely recover their turgidity (Glover 1959)

Stomatal aperture is governed by an osmotic imbalance between the guard cells and their surrounding cells, the first response of the stomata to onset of water stress is to open slightly but only temporarily. Since the guard cells are not in immediate contact with the mesophyll, water is withdrawn from the surrounding epidermal cells before it is withdrawn from guard cells. As a consequence, the surrounding epidermal cells become relatively flaccid temporarily, allowing the guard cells a turgor advantage which results in the opening of the stomatal pore. This opening is only transient and as water stress continues, water passes out of the guard cells to the mesophyll cells via the surrounding cells. The sensitivity of stomata to a decrease in leaf water potential varies between species and is influenced by age and growth conditions of plants (Jordan and Ritchie 1971; McCree and Davis1974; Turner and Begg 1973). Turner (1974) has shown that when stomatal response is related to

turgor rather than to water potential, there is a smaller difference between the apparent sensitivities to stress of stomata of different species.

1.2.4 The stomatal complex and its development

The stomata develop from the protoderm, the mother cell of the guard cell is the smaller of two cells resulting from an unequal division of a protodermal cell (Bunning and Biegert 1953; Bonnett 1961). The small guard mother cell (GMC) divides into two guard cells which through differential expansion acquire their characteristic shape.

Whilst stomata are differentiated from protodermal cells early in development of plant organs, differentiation continues for some of the time over which the organ grows. The expansion of leaf results mainly from the division of marginal and intercalary meristematic cells leading to the differentiation of the epidermis including the stomata and other tissues. Cell enlargement follows cell division and, therefore, counts of stomata per unit area carried out at different stages in leaf development will differ, tending in the earlier stages to be higher than in later stage. Individual stomata, once differentiated, grow in size and may change in shape as the leaf blade expands. An ontogenetic system of classification of stomata based primarily on the mode of division of the guard mother cell with respect to orientation of the wall which cuts it from the meristemoid has been suggested by Payne (1979).

Basically, stomatal complexes can develop in three different ways (Martin *et al* 1983):

Agenous development The stomatal meristemoid divides symmetrically once to form a pair of guard cells. No subsidary cells are present.

Mesogenous development. The stomatal meristemoid divides more than once to produce a guard cell pair and at least one subsidiary cell is produced

Perigenous development as in the agenous pathway, the stomatal meristemoid under goes a single symmetrical division to form the guard cell pair.

Many types of plants can be classified on the number, shape and distribution of subsidiary cells. Bryophytes are agenous, pteridophytes are mesogenous or rarely agenous, dicotyledons are either mesogenous or genous and monocotyledons are either perigenous or agenous. The differences are probably related to the position of the leaf meristem which is typically basal in monocotyledon and apical and lateral in most other plant groups. The subsidiary cells do not arise directly from the meristem but they usually develop from protodermal cells adjacent to the guard mother cells, but they may also develop from sister cells of the mother cell, this was observed by De Barry (1877) (as quoted by Fahn 1974) who reported one of the earliset studies on stomatal complex formation. Many types of stomatal complexes can be classified on the basis of the number and position of the subsidiary cells and on the ontogeny of the cell types (Baranova, 1987)

The formation of guard mother cells and subsidiary cells usually involves asymmetric division in which the nucleus migrates to a specific site in the parent cell before dividing (Hepler and Palevitz 1974). In addition, the orientation of the new cell plate is often markedly different from that resulting from the division of other epidermal cells. The development of the epidermis of the aerial parts of the plant is principally related to the limitation of water loss, epidermal cells thus have an identifiable and specialized structure. Division of an epidermal cell may therefore result simply in the production of a new cell of the same type or a more specialised cell, e.g a stomata or trichome.

The distribution of stomata is a simple and accessible example of biological patterning. The epidermis is a two dimensional structure in which the distribution of the stomata is more spaced or orderly than would be expected for a random distribution. The pattern has been attributed to three different control mechanisms:

(a) Stomata could be spaced because each stoma inhibits similar initiation in neighbouring cells (Bunning and Sagromsky 1948; Korn 1972, 1981; Meinhardt 1982).

(b) The distribution of stomata could be a reflection of an early pre-pattern of morphogenetic determinants of the cell differentiation (Meinhardt 1982).

(c) The cell lineage that forms the stomata also forms the surrounding cells (Bunning and Sagromsky 1948; Sachs and Benoualche 1974, 1988). Thus,

stomata could become separated by a minimum number of cells even though the locations of the initial events of their formation were random.

The development of stomatal complexes has been examined extensively in relation to their cellular derivations and final morphological form and has been reviewed by Fryns-Claessens and Van Cotthem (1973), but there is little information available concerning the structure of the guard cells during their development from protodermal origins. Stebbins and Shah (1960) examined the stomata from several types of plant where the functional complex was composed of between two to eight cells. The ontogeny of the guard cells is the outcome of a complicated process, including:

(1) a number of divisions, the first one(s) of which are differential and sometimes obviously asymmetrical and the last of which always results in the symmetrical formation of a pair of guard cells (Bunning and Sagromsky, 1948; Bunning and Biegert, 1953; Stebbins and Shah 1960; Stebbins and Jain, 1960).

(2) a differentiational sequence in the young guard cells expressed structurally by conspicuous protoplasmic changes, the most prominent being related to microtubule organization, intense dictyosome activity, and plastid differentiation (Lander, 1969 a&b, 1970, 1972; Kaufman et al 1970; Srivastava and Singh 1972; Singh and Srivastava 1973).

Stomatal guard mother cells embark on a highly specific course of development that differs markedly from that of surrounding vegetative initials that will form lamina cells of the leaf epidermis. The mature guard cells

surround a central pore and often a specific number and pattern of accessory cells are associated with them. In some cases, GMCs control the formation of subsidiary cells by somehow including polarized mitosis in neighbuoring cells (Stebbins and Shah, 1960; Pickett-Heaps and Northcote 1966; Palevitz 1981; Galatis *et al* 1983).

In the mature epidermis of some plants there are structures which represent immature stomata (Sachs and Benouaiche 1978; Charlton 1988). The presence of these structures might indicate the operation of another class of developmental control: patterning could be the result of a random initiation of an excess number of potential stomata resulting in patterned or spaced mature stomata (Sachs 1988,1991). Growth and differentiation in guard cells is characterized by a change in shape and patterned deposition of wall thickenings. Cellulose microfibrils are precisely oriented and associated with an underlying system of co-oriented cortical microtubules (Palevitz 1981). In the highly coordinated development of stomata, changes in one of the guard cell pair is mirrored in the other. The differentiation and fine structure of the guard cell and subsidiary cells in *Avena*, after aldehyde fixation, has been reinvestigation by Kaufman *et al* (1970).

Light microscopy has yielded considerable information on development of stomata in grasses (Campbell 1881; Porterfield 1937; Flint and Moreland 1946; Stebbins and Khush 1961; Arivanayagam and Stebbins, 1962; Kaufman and Cassell 1963; Kaufman *et al* 1969). Less is known at the ultrastructural

level. However, Pickett-Heaps and Northcote (1966), and Burgess and Northcote (1967) have shown preprophase microtubule bands early in subsidiary cell and guard cell formation. These workers suggest that these bands, coupled with nuclear migration in long cells, indicate that a particular short cell in the protoderm will become a stoma mother cell. Others (Brown and Johnson 1962; Pickett-Heaps 1967) have clearly demonstrated that pores occur in the common wall between guard cells at the ends of these cells in mature stomata. The kind of organelles that occur in mature guard and subsidiary cells have also been described (Setterfield 1957).

Stomatal complexes in wheat (*Triticum aestivum*) consist of four cells, two guard cells and two subsidiary cells. The epidermal cells from which the complex develops are partially differentiated, being vacuolate. Prior to the division which initiates stomatal differentiation,the nucleus of the epidermal cell migrates to one end to give a polarized structure. The cell then divides in this asymmetric configuration, to give rise to two daughter cells which are quite different in appearance and size. The future guard cell is small and does not contain a large vacuole. The other daughter cell, which is destined to give rise to a subsidiary cell, is highly vacuolate and immediately following the division is polarized in the same sense as the original epidermal cell. As this cell progresses toward division, its nucleus migrates and comes to rest against that part of the wall which is common with the guard mother cell of the adjacent row in the epidermis. The cell plate which partitions the cytoplasm between

these two cells at cell division is curved. The final stage in the development of the complex is signalled by a symmetrical division of the guard mother cell. This gives rise to the two guard cells, which are similar in size and structure and which remain partially connected together through large spaces in the cell plate. The functioning state of the stomata is attained by thickening of this cell plate in its central region followed by splitting of the thickened wall to produce the pore.

1.2.5 Guard cells

The term guard cell is entirely appropriate since these cells which show rapid changes in turgor function to protect the moist underlying tissues from extremes of dehydration. The initial development of guard cells is identical in all the plant groups with the immature guard cell pair fused together by their ventral walls. The principal changes which occur during the physical maturation of guard cells are the differentiation of the cell walls and the development of the pore. During their development the middle lamella between the two guard cells swell and become lens-shaped shortly before the time when it breaks down to form the stomatal aperture (Ziegenspeck 1944; Galatis and Mitrakos 1980). Martin *et al* (1983) suggested that, whilst aperture formation is initiated enzymatically, the separation of the two guard cells is brought by osmotic force driven from starch hydrolysis within the cells themselves.

Guard cells undergo radical changes in shape as they grow; that is, they transform from rough cylinders to cells with characteristic shapes such as the kidney-like cells of *Allium* (Bunning and Biegert 1953; Stebbins and Jain 1960) and the bone-shaped cells of grasses (Stebbins and Shah 1960; Kaufman *et al* 1970, Srivastava and Singh, 1972). These changes are accompanied by the deposition of new, highly-oriented wall microfibrils which have characteristic thickenings at specific sites in the cell (Ziegenspeck 1944; Singh and Srivastava, 1973). There is no radical change in shape of the guard cells as they grow in size except that the early rounded shapes change into a more elongated, elliptical one; as in most dicotyledons and the dumbell-shaped as in most monocotyledons.

In positioning guard cells of stomata may be level, sunken or raised in relation to the other epidermal cells. Below the stomata are large intercellular spaces which are termed sub-stomatal chambers. Guard cells not only differ from other epidermal cells by their morphology and anatomical features, but also in their cytoplasmic inclusions, vacuolar properties and, indeed, their metabolism, especially as effected by light. Chloroplasts do occur in the epidermal cells of some leaves, but the presence of chloroplasts in practically all guard cells is a significant feature. Guard cell chloroplasts are especially rich in starch.

Stomatal characteristic are of interest in plant development, physiological studies and in the development of cultivars to improve productivity particularly under stress conditions.

1.2.6 Environmental influences on stomatal pattern development.

It well known that stomatal number varies among species (Hirano 1931; Meidner and Mansfield 1968). The numbers of stomata per square millimeter differs from species to species. For the abaxial leaf surface of *Oxalis acetosella* Martin and Juniper (1970) reported that there were 37 stomata per square millimeter. In other studies (Meyer and Meola 1978) reported much higher numbers for other species; 402 for *Quercus calliprinos*, 545 for *Olea europaea*, and 1,198 for *Quercus lyrata*.

Whilst specific stomatal numbers have been reported for many plant species their values are not always fixed under all environmental conditions. Results of a number of studies indicate that there is considerable plasticity in the formation of the stomatal complement in leaf tissue. Use has been made of two major parameters to describe stomatal patterns. Stomatal density/ frequencies are used to describle the simple number of stomata per unit area of leaf, however, these measurements are subject to variablity related to epidermal cell size (Meidner and Mansfield 1968). An increase in overall cell expansion in a leaf epidermis can bring about a reduction in density and is related directly to

water supply. In contrast the stomatal index, the number of stomata expressed as a proportion of total epidermal cells, is not related to cell size, and hence water uptake, but is a developmental expression (Salisbury 1927). This measurement gives a more meaningful indication of environmental influences on stomatal development.

1.2.6.1 Influence of light

Light intensity is an important factor in determining the morphological and anatomical structure of plants. One of the anatomical characteristics which may be simply expressed quantitatively is stomatal density. Early observations showed that the leaves of plants, growing under higher light intensity, have higher stomatal densities (Rea 1921; Goette 1932). Pazourek (1970) found in *Iris* plants grown under different light intensites that stomatal frequency decreased with increasing light intensity. However, he reported the total stomatal number to be constant at only higher light intensities, with a decrease in total stomata number at lower light intensities. In contrast, experimental cultures of *Sambucus nigra*, *Fagus sylvatica*, and *Vaccinium myrtillus*, used by Salisbury (1927), showed significant differences in stomatal frequency corresponding to differences in illumination. Kubinova (1991) also found no significant difference in foliar stomatal frequency of barley exposed to different light conditions.

Many of the influences of light on plant developmental processes involve alterations to cell expansion. This is turn will influence the density of stomata. For example in spring varieties of wheat an increase in light intensity over the range 200 to 5000 ft-c reduced the length of the lamina by reducing both the number and length of epidermal cells (Friend *et al* 1970). In similar studies on the effect of light intensity Friend *et al* (1962) reported that the laminae formed on plants grown at 2500 ft-c were shorter than those grown at 500 ft-c. This effect of light was related to both a reduction in cell expansion and a reduction in the number of cell divisions along the lamina (Friend 1966).

Where stomatal index has been measured, Schoch *et al* (1980) found that both light intensity and any variation in this light supply will affect this parameter. However, he found that the stomatal index in *Vigna sinensis* L. was not constant as suggested by Salisbury (1927). In contrast, Rahim and Fordham (1991) reported, for garlic cultivars, that the stomatal index decreased with increasing light intensity. The amount of light energy received by a leaf in which stomatal differentiation occurs is not the only factor which determines the number of stomata produced per unit area. The distribution (bright and shade) of light during ontogeny is another factor that must be considered (Schoch 1972).

1.2.6.2 Influence of carbon dioxide

One of the most generally accepted long term atmospheric changes is the increase in carbon dioxde concentration (Penuelas and Matamala 1990). Growth and development of plants are affected by CO_2 and interspecific differences in these responses are well documented (Mott 1990; Sasek and Strain 1991; Woodward *et al* 1991).

Variations in atmospheric CO_2 concentrations have occurred throughout the Earth's history (Gammon *et al* 1985). Evidence from anlaysis of air trapped in Antarctic ice suggests that over the past 200 years, the CO_2 has risen far more rapidly than in the past, to the current ambient level of 340 p.p.m.v, due mainly to the combustion of fossil fuels during the industrial area (IPCC, 1990). In addition to overall effects on growth, rapidly rising CO_2 concentrations may induce more specific changes for example in leaf stomatal densities which in turn control maximum values of stomatal conductances (Eamus *et al* 1993; Berryman *et al* 1994). Ferris and Taylor (1994) have reported that stomatal density responds to CO_2 concentration over the range from c. 345-590 $\mu mol\ mol^{-1}$, supporting earlier observations on individual species (Madsen 1973; Oberbauer *et al* 1985; Woodward 1987; Woodward and Bazzaz 1988; Beerling *et al* 1992). In response to CO_2 enrichment, some species show decreases whereas others increases and others no changes in stomatal density (Beerling 1993; Rowland *et al* 1990; Woodward and Bazzaz

1988). However, Clifford *et al* (1995) suggested that the effects of future increases in atmospheric CO_2 on stomatal frequency in groundnut are likely to be small, sepecially under conditions of water stress. More importantly, Woodward (1987) showed that changes in stomatal density were paralleled by changes in the stomatal index, indicating that the effect of CO_2 was potentially on stomatal initiation, rather than an indirect effect mediated through changes in the rates of leaf cell expansion. Many studies, however, have shown that for CO_2 exceeding the current ambient levels of 353 μmol mol^{-1} neither stomatal density (O'Leary and Knecht 1981; Woodward 1986; Ryle and Stanley 1992) nor stomatal index responded (Thomas and Harvey 1983; Mousseau and Enoch 1989; Ragdoglou and Jarvis 1990). Recent studies (Ferris and Taylor 1994), however, have shown that CO_2 has a contrasting effect on stomatal index of four native herbs. Historical trends in stomatal density changes, derived from examinations of macrofossils, are also conflicting (Beerling *et al* 1991; Beerling *et al* 1992).

1.2.6.3 Influence of water status

In addition to effects on stomatal function, long term exposure to water deficit may have developmental effects on guard cell size, frequency and the stomatal index (Quarrie and Jones 1977; Spence *et al* 1986). Penfound (1931) observed that fewer stomata per unit area were present in plants growing under

'optimum' soil moisture conditions Water stress can increase stomatal density, but reduced stomata size and area so that the area of the stomata apparatus per unit leaf area or the number of stomata per leaf remains unchanged. In a study of *Sesleria caerulea* L (Ferris 1991) found a significant decrease in stomatal index of the leaf with increasing water deficient. These effects could also reduce photosynthetic efficiency, and hence growth. The mechanisms and magnitude of responses to water deficit are as yet not fully characterised and relatively uninvestigated.

Clearly the effect of drought stress on stomatal density is important for the establishment of plants under arid conditions. Here water use efficiency is related to total stomatal porosity in the leaf. It would be of selective advantage to plants growing in areas of water limitation to be able to adjust stomatal number and size to suit the prevailing hydrology.

Aspects of this form the basis of this research project where the influence of water limitation has been examined on wheat, a plant of mesic environment, and sorghum, a plant of more arid zones.

1.3 Wheat

The wheat plant is the oldest of man's crop. A large amount of variation has developed in the crop, so far some 17,000 different varieties have been produced. Most modern wheat is the hexaploid, *Triticum aestivum* L, and is

the most abundant by far. The tribe Triticeae is economically the most important group of the family Gramineae. It has given rise to cultivated wheats, ryes and barleys. Wheat (*Triticum*) and ryes (*Secale*), together with the genera Aegilops, Agropyron, Eremopyron and Haynaldia, are in the subtribe Triticinae. This subtribe is relatively young and therefore hybridization between individuals of different genera can still take place and result in either direct exchange of genetic material or amphiploidy.

The wild diploid species have diverged considerably from each other. This divergence is particularly evident in the morphologically well-defined seed dispersal mechanisms, their specific ecological requirements and geographical distributions. Cytogenetic data have corroborated the taxonomic classification by showing that each diploid contains a distinct genome (Kihara 1954). Hexaploid *T. aestivum*, probably originated and entered cultivation only after the more-or-less simultaneous domestication of diploid and tetraploid forms. It appears in archaeological data from the seventh millennium BC.

T.aestivum is known to have occurred in the Middle East at least as early as 5800 B.C (Helbaek 1966), and subsequently spread from there around the Mediterranean and through central Europe to higher latitudes and more humid environments (Waterbolk 1968). The wheat plant has been comprehensively described by Percival (1921) and aspects of its physiology have been discussed by Peterson (1965), Quisenberry and Reitz (1967) and Wardlaw (1974).

Germination of wheat caryopses will occur between 4 and 37°C, with 20-25°C being the optimal. The minimum moisture content for germination is 35-45% of grain dry weight, and germination is more rapid as moisture increases above this level. Light is not of great importance in controlling germination of wheat (Grahl 1965). Germination can occur at a relative humidity of 97.7% which is below the permanent wilting point for the growing plant (Owen 1952) and as the seedling develops it becomes more susceptible to water deficiency (Milthorpe 1950).

The growth of the root may exceed shoot growth at low temperatures but as temperature rises the growth of shoots increases more than that of the roots (Brouwer 1966). This difference may result from increased competition for assimilates between root and shoot at higher temperatures (Friend 1966; Wardlaw 1968). Shoot growth is dependent on root function and the role of roots is not confined to nutrient and water uptake. Cereal roots can reduce nitrate (Miflin 1967), but most nitrate reduction probably occurs in leaves in the light (Stoy 1955; Minotti and Jackson 1970).

The rate at which leaves are initiated from an apical meristem,emerge and unfold, as well as the shape and size of the mature lamina, depend on the temperature, light intensity, day-length and nutritional status under which the plant is grown. With constant light and temperature conditions Friend *et al* (1962) obtained a maximum area per leaf at 10000-19000 lx and 20°C. The

temperature for maximum width is lower than that for maximum blade length (Chonan 1971). Of particular importance is the degree of water limitation on the plant which will have marked effects on water availability for cell expansion, and on primary productivity through metabolic effects.

1.4 Sorghum

Sorghum is the fourth most important of the world's cereals, coming after wheat, rice and maize. It is grown in the semiarid regions of the world. The greatest variability in both cultivated and the wild sorghums is found in the north-east regions of Africa. Sorghum belongs to the tribe Andropogoneae; this tribe probably originated in tropical Africa. The Sorghastrae is one of the sixteen subtribes of the Andropogoneae, (Garber 1950, 1954 and Garber & Snyde 1951) split the subtribe into two main genera, and then subdivided the genus sorghum into six subgenera. Sorghum section *Sorghum* has a polymorphic diploid population in tropic Africa which is referred to as *S.arundinaceum*, and a distinctly different diploid population in Southeast Asia, Indonesia and the Philippines, *S.propinquum*.

Sorghum is a short-day plant, and most varieties require fairly high temperatures to make substantial growth. The crop can withstand periods of drought better than can most cereals and tolerates a wide range of soil conditions. It can grow well on heavy clays, especially some of the deep cracking valley-bottom and balk cotton soils of the tropics. At the other

extreme, good crops can also be grown on light, sandy lands as in the Arabian peninsula. Soil acidity is seldom important, and sorghum crops can be grown within a soil pH range of 5.0 to 8.5. It also tolerates salinity better than does, for example, maize (Hayward and Bernstein 1958). Sorghum grain is used for stock feed in Japan and in Europe but provides human food in India and in Africa. It is also used for fermentation in Africa to make alcoholic beverages.

The straw is fed to livestock and is used for fencing, while the plant structure provides fuel for cooking. Under drought stress, hydrocyanic acid may be produced in the foliage, which can then poison livestock. Some sorghums have sweet juicy stems which may be chewed or used to produce syrup.

Sorghum can be grown with some degree of success anywhere in the world where the summer temperature averages 65°F or higher, with a frost-free period of 120 days or more, and with an arable soil supplied with sufficient moisture to grow any field crop. Vinall and Reed (1918), as the result of a series of studies and observations, concluded that the optimum temperature for sorghum growth was about 33°C or 34°C. Sorghum varieties cannot tolerate low temperatures,16°C being near the minimum for most types (Rhykerd *et al*, 1960). Freezing effects may be important for determining the growing period for sorghum, temperatures only slightly below freezing point will kill sorghum plants at most growth stages, although some young plants may withstand a

slight frost in the first three weeks of life (Martin 1941). The grain can survive low temperatures down to -7°C without apparent damage provided that the moisture content is below 15% (Robbins and Porter 1946; Gritton and Atkins 1963a).

Grain sorghum requires large amounts of essential minerals for satisfactory yields. However, sorghums survive and produced satisfactorily yields under a wider range of conditions. Martin (1930) drew attention to the slower wilting of sorghum than of maize under conditions of water deficit, and of its powers of recovery after wilting. Glover (1959) showed that this distinction was of fundamental importance. He found that maize leaves subjected to wilting for a week or more suffered permanent damage to the stomata. The adaptation of sorghum especially the grain types, to dry climates is well known; the reason for such an adaptation is less well known. Sorghums have many secondary roots per unit of primary root, this fact may allow the sorghum to be more efficient in water absorption.

The sorghums have the ability to become practically dormant during periods of severe droughts and to renew growth, without much apparent injury when conditions become more favourable. Drought conditions cause their leaves to curl up and form a closed tube that shields the stomata and reduces transpiration.

1.5 Aims.

The number of stomata in the leaves of crop species is of the ultimate importance in the control of the balance between photosynthetic carbon fixation and water loss. This feature is of most importance when plants are grown in conditions of reduced water limitation. In addition to effects on stomatal function, water limitation may also influence the degree of development of the stomatal complex which in turn will influence a water / CO_2 balance.

This project was devised to :-

1   Investigate the effects of environments factors, water limitation in particular, on stomatal development in seedlings of two cereals. Wheat was chosen as plant from mesic conditions with sorghum being a plant from more arid zone conditions.

2   Investigate the early stages of determination of the guard cell in epidermis of the leaves and the influence of environmental factors on it.

3   Investigate the potential signals from roots to the leaves which could influence stomatal development.

4   Investigate the development of stomata using a coleoptile system model.

5   Investigate potential plasticity between stomata and trichome production in relation to water limitation.

CHAPTER 2
MATERIAL AND METHODS

2.1 Biological material

Caryopses of wheat *Triticum aestivum L* genesis winter, and Zea maize campus spring were supplied by the Biological Sciences Department at the University of Durham, England (UK). Different varieties of *Sorghum bicolor* (L.) Moench; were obtained as follow :-

Funk, Morgon 854, Morgon 856, and Pioneer 8ss from Argentina.

Saudi Red from Gizan province Saudi Arabia.

Indian White imported into Saudia Arabia from India.

Wheat caryopses and sorghum seeds were stored dry at room temperature.

2.2 Plant growth conditions

Caryopses of wheat were soaked in distilled water for 24 hours before being transferred to moist paper toweling in a plastic tray. The trays were maintained in the dark at 25°C under a relative humidity (RH) of 100% from automatic watering sprinklers until germination took place. After germination, wheat and sorghum seedlings were transferred to a growth room at a constant temperature of 25°C with a photoperiod of 16 hours at a light intensity of 100 μmol photon m^{-2} s^{-1} PAR, supplied by banks of Phillips Warm White fluorescent tubes. Dark period

temperatures were ambient, stabilised usually around a minimum of 15°C without supplementary heating. The plants were left to establish for seven days before stress treatments were applied.

2.3 Seedling cultural conditions

Two experimental systems were used to restrict water availability; one a pot growth system and the other a hydroponic system.

2.3.1 Soil growth conditions

Upon radical emergence seedlings were individually transferred to 10 cm x 7 cm pots. The pots were filled with Levington compost mixture and were kept under controlled plant growth room conditions as above. They were then left to establish for 7 days before three regimes of water treatments were applied to each species for a minimum of twenty days.

1. 50 ml of water every day per pot (Control)

2. 50 ml of water every 4 days per pot

3. 50 ml of water every 7 days per pot

2.3.2 Honey jar cultural conditions

After germination, uniform wheat seedlings were selected and were grown on in glass honey jars. The jars had a volume of 500 ml and the seedlings were

inserted through holes in a plastic lid being held in place by cotton plugs as shown in Plate 2.1. The glass containers were covered with aluminum foil to prevent algal growth. After two days growth in water the seedlings had adjusted to the culture conditions. At this time the lids and plants were removed and the water in the jars was replaced with Evans and Nason's culture solution. In both cases seedlings were harvested after 20 days, by which time the fifth leaf was fully-emerged as judged by the emergence of the ligule. Aeration was obtained by bubbling air from a plastic tube attached to an aquarium pump and by regular changing of the solutions.

Plate 2.1 Wheat plants growing in honey jar hydroponic culture.

2.3.2.1 Specific treatments of seedlings

2.3.2.1.1 Nutrient solution composition

The stock solution was prepared from nutrient elements as described by Evan and Nason (1953). Table 2.1 shows the proper concentrations of the elements. The solution was adjusted to pH 6.0 using sodium hydroxide. The stock solutions were kept in the refrigerator at 4.0°C and were renewed every 3 months. For experimental treatment purposes the nutrient solution was replaced with one of specific composition. When necessary sorbitol was added at g/l (M) and mannitol at g/l (M). Detals of levels of PEG and abscisic acid are gave in 2.3.2.1.2 and 2.3.2.1.3 respectively.

Table 2.1 Composition of mineral salts in the Evan and Nason growth medium used to raise wheat plants (Evans and Nason 1953).

Salts	Concentration
$Ca(NO_3)_2.4H_2O$	0.005 Molarity
K_2SO_4	0.0025 "
KH_2PO_4	0.0005 "
$MgSO_4.7H_2O$	0.002 "
Fe-EDTA.	0.5 Fe Mg l^{-1}
KCl	9.0 Cl "
$MnSO_4$	0.25 Mn "
H_3BO_3	0.25 B "
$ZnSO_4$	0.25 Zn "
$CuSO_4$	0.02 Cu "
Na_2MO_4	0.02 Mo "

Wheat plants grew and developed in this basic salts medium.

2.3.2.1.2 PEG treatment of seedlings

Seedlings were grown in hydroponic culture, with added PEG (6000), from the time of emergence of the coleoptile. PEG was initially used in a series having a gradation in osmotic concentrations. This was prepared by dissolving the

appropriate amount of PEG in nutrient solutions at concentrations of 50, 75, 100, 125, 150 and 200 g/l. As matter of routine two concentrations only of PEG were used for most of the experiments, 125g/l and 200g/l.

2.3.2.1.3 Abscisic Acid (ABA)

In order to study the consequences of ABA on stomatal development in wheat seedlings the nutrient solution in the glass jars was replaced with one containing the follow concentrations of ABA 1.0, 0.1, 0.01 ppm prepared from a 5 mg/l (M) stock stored at 4°C. ABA treatment was given over a 10-day period prior to harvesting and examination of the stomatal patterns.

2.3.2.1.4 Sodium chloride

Initial experiments were performed where wheat caryopses were germinated in Petri-dishes containing five NaCl concentrations (10, 20, 50, 100, 200 mM). It was noted the two highest concentrations were lethal to plants, therefore, for root growth experiments the concentration of 50mM was used in the honey jars, dissolved in nutrient solution.

2.4 Plant growth characteristics
2.4.1 Leaf elongation

Growth during the PEG treatment was expressed in terms of the leaf length which was recorded also for about 1 to 2 days prior to stress incubation. The length

of all leaves, up to the 5th, was recorded as they emerged every two days over at least a 20 days development period.

2.4.2 Root and shoot dry weight

Roots and shoots were separated, seedlings were harvested from honey jars at the end of the incubation periods, blotted dry and transferred to small crucibles, for fresh weight determination. The crucibles were then put in an oven at 80°C overnight and were then cooled in a desiccator for at least 30 min before the weight was recorded again. The weight of the plant material was obtained by subtracting the weight of the crucibles from the weight of the crucibles plus the roots or shoots.

2.5 Split root experiments

In order to assess the effects of different growth conditions an half roots a split root system was adopted. For this seedlings were maintained in honey jar cultivation, using culture solution without added PEG. After 3 days the seedlings were removed from the jars and used for split root cultivation. Seedlings were taken and arranged so that each half of the complete root system was immersed in culture solution in individual glass boiling tubes which were placed either side of a center partition of a H-shaped glass tube, as in Plate 2.2. Seedlings were grown on with one set of roots in nutrient solution with added PEG 125g/l and the other in nutrient

Plate 2.2. Arrangement for growing wheat plants in a split root configuration.

Half roots were inserted through glass tube stems from the upper container into the boiling tubes.

solution without added PEG. Seedlings were harvested after twelve days of growth, by which time the third leaf was fully emerged and this was used for calculation of the stomatal index as described later.

2.6 Anatomical observations

2.6.1 Plant tissues

The third leaf was selected for studies since this emerged in plants after they were subjected to stress conditions. Four samples for each concentration were selected for study.

2.6.2 Fixation of the sample

The material was fixed in immunofixative solution containing 50 ml paraformaldehyde, 10 ml glutaraldehyde, 10 ml PBS and 130 ml distilled water. The solution was adjusted to pH 7.0 with concentrated H_2SO_4. Fixation was allowed. for at least 48 hours at 4°C.

2.6.3 Dehydration

Fixed samples were dehydrated in an ethanol series as follows:-

Ethanol 25% , 40% , 60% , 75% , and 95%, half an hour in each solution one hour in 100% then overnight in fresh 100% ethanol.

2.6.4 Infiltration and embedding

Following overnight dehydration specimens were incubated as follows:

Overnight in LR white : Dry ethanol (1:3), then

Overnight in LR white : Dry ethanol (1:1), then

Overnight in LR white : Dry ethanol (3:1), then

100% LR white for 2 days until infiltrated.

Finally the specimens were placed in the centre of 100% LR white (Medium Grade Acrylic Resin) in flat-based TAB polypropylene capsules. The capsules were incubated overnight in an oven at 57-60°C to polymerise the resin.

After embedding of the material, sectioning was carried out using a Sorvall MT2-B Ultra Microtome. The sections were stained with 0.1% Toluidine blue until dry on a hot plate before being examined under a microscope.

2.6.5 Microscope examination

A Nikon Diaphot-TMD inverted microscope with TMD-EF epifluorescence provided with violet (V), blue violet (BV), ultra-violet (UV) excitation filter cassettes, was used. Photographs were taken using a Nikon camera with ASA 100 Fujicolor film.

2.7 Coleoptiles

2.7.1 Tissue growth

Caryopses of wheat were soaked overnight in water and germinated on moist filter paper (Whatman No 1) in Petri-dishes in dark at 25°C. After four days of growth whole seedlings (caryopses, coleoptile and roots) were harvested.

2.7.2 Tissue treatments

2.7.2.1 PEG

Two experiments were conducted to study the effect of PEG on stomatal development on wheat coleoptiles.

2.7.2.1.1 Petri-dish experiments.

Three sets of tissues were used :- Whole seedlings, seedlings without roots and seedlings without the caryopses and. Each set of seedlings were incubated in Petri-dishes in the dark room at 25°C whilst bathed in treatment solution. All excisions were made under the surface of the treatment solution in order to prevent cavitation. After 4 days of growth 2 cm sections were removed from the coleoptile with a double-bladed cutter and mounted on a slide with a drop of 1% Iodine prior to examination under a light microscope. One section was the top 2 cm of coleoptile whilst the other was the middle 2 cm. In some experiment only the top 2 cm region was examined as this contained more stomata.

2.7.2.1.2 Honey jar experiment

After three days from the initiation of sowing, the whole seedlings (with the roots and caryopses) were placed on gauze fixed over glass honey jars of nutrient solution. The seedling roots were placed through the gauze holes into the nutrient solution. The jars were then kept in the dark for 2 days before treatment commenced. The jar nutrient solutions were replaced with 125g/l PEG solution as required. Coleoptiles were harvested 4 days after application of the stress and examined as above.

2.7.2.2 ABA

In order to study the consequences of ABA on stomata mother guard cell in formation and differentiation wheat coleoptile, three concentrations of the growth regulator (1.0 ppm, 0.1 ppm and 0.01 ppm) were chosen. Caryopses were grown under similar conditions in the dark growth room as already described. After 3 days the whole seedlings were transferred to the honey jars as in 2.7.2.1.2. The nutrient solution was replaced after 2 days with one containing ABA. After 4 days, 2 cm sections of each coleoptile under different treatments were selected for studies. The sections were stained with calcaflour 1% (w/v) and examined under a light microscope with an ultra-violet filter.

2.7.2.3 Light

Ten pre-soaked caryopses were placed in Petridishes on filter paper moistened with nutrient solution, two Petri-dishes were placed in light growth room under same conditions mention before, the other Petri-dishes were wrapped with two layers of aluminum foil and were kept in the dark room, up to the end of the experiment. After one week of growth, the coleoptiles were harvested and fixed in a solution of formyl saline until required for examination. The enclosed first leaf was not removed from the coleoptiles in order to simplify the procedure and minimize the damage in handling the sections.

2.8 Stomatal characteristics

Two methods were used in this study to obtain stomatal / epidermal cell counts. Leaves of similar sizes, for each species were used and comparable portions of the upper and lower surfaces of the leaves were chosen for examination.

2.8.1 Nail varnish

Replicas of the leaf surface were made by spreading a thin layer of nail varnish (Boots No 17) on to the abaxial and adaxial surfaces of each leaf in sorghum varieties and on adaxial surface in wheat leaves. The parts of the leaf used in this

study were top, base and sheath for each species and four leaves (each from an individual plant) were used.

After being peeled from the leaf the varnish copy was placed on to a microscope slide and covered with a cover slip. The leaf surface replicas were examined under a light microscope to obtain the total number of stomata, epidermal cells and trichomes for the calculation of stomatal density, stomatal indices and trichome indices of the leaves (see below).

2.8.2 Ethyl acetate impressions

A leaf section was placed on a small square of acetate sheet which in turn was placed on a microscope slide. A few drops of absolute acetone were added by pipette to the acetate sheet, and the leaf section. The leaf section was heavily weighted down for about five minutes under a second microscope slide, and was then gently peeled away, leaving an impression on the acetate. This was then used for microscope examination as above for the nail varnish peels.

2.8.3 Stomatal density

The number of stomata and the number of other epidermal cells were counted at x400 magnification from five whole fields of view (area 0.0908 &

0.0804mm^2) from each of four individual leaf surface peels (total 20 fields). The number of stomata were converted to number of stomata / mm^2.

2.8.4 Stomatal index

From the total epidermal cell count data the stomatal index was calculated which relates the number of stomata per unit area (S) to the number of epidermal cells per unit area (E) where the stomatal index (SI) = [S / (E + S)] x 100. (Salisbury, 1928). This is the proportion of stomata to overall epidermal cell number.

2.8.5 Stomatal pore size

Stomatal pore lengths were measured under the same magnification from five fields of view on each of four leaves giving a mean length from 20 measurements. A calibrated microscope slide was used to convert measurements to micrometers.

2.8.6 Trichome index

The trichomes as a percentage of total epidermal cells was calculated as for the stomatal index.

Trichome index (TI) = [T / (E + T)] x100

2.9 Root pigmentation

Root pigmentation was observed during treatments of wheat plants with PEG. The nature of the pigment was investigated using the following methods.

2.9.1 Extraction of the pigment

Plants were grown in nutrient solution with PEG (200g/l & 125g/l) added to the media for fourteen days under similar conditions in the growth room as described earlier. Plant roots were harvested upto 16 days after application of the PEG stress treatment, they were frozen in liquid nitrogen and stored at -70°C.

Approximately 0.7 g of plant roots were homogenized in a mortar and pestle in 10-15 ml of cold (-20°C) absolute ethanol or diethyl ether with a small amount of sand. The residue was filtered through filter paper (Whatman No 2) and re-extracted in a further 10-15 ml of ethanol or diethyl ether. Following a second filtration the pooled filtrates were reduced to dryness using a rotary film evaporator.

2.9.2 Purification of the pigment by Thin-layer Chromatography (TLC).

The pigment sample was redissolved in 0.5-1.0 ml of ethyl acetate, and was then transferred to two Eppendorf tubes and reduced to dryness in a vacuum desiccator. The sample was further redissolved in 20-30 µl of ethyl acetate and was

centrifuged for 2 minutes to concentrate any residual non-pigment material in the bottom of the tube. Spot samples of the solution of the pigment were placed 2 cm from the bottom edge of a Whatman Al SIG TLC silica plate. A hair dryer was used to concentrate the spots. The plate was then developed in a glass TLC tank containing the developing solvent; diethyl ether, hexane:diethyl ether (9:1) or absolute methanol as required. After ca. 45-60 min the plate was removed from the tank and it was permitted to air dry in the fume hood for a few minutes. The spots were examined under ultraviolet light (254, 300, 330nm) for fluorescing compounds. Two-way TLC was performed by allowing the plate to develop in one dimension before being dried and returned to the second solvent having been rotated through 90°.

2.9.3 Spectrophotometric analysis

A Beckmann Instrument DU-7500 Spectrophotometer was used in this study. The pigment spot regions were scraped from the TLC plates using a spatula and the silica was transferred to an Eppendorf tube together with 1.0 ml of ethanol or diethyl ether.

The tube was then left on ice for 30 min and then centrifuged for 5 min at 40°C. The supernatant was transferred to a second tube and analysed spectrophotometrically at wavelength range from 250-500 using ethanol or diethyl

ether as the blank reagent. A recording of the spectrum was taken. All absorption spectrum traces have been copied from the originals. (NB for Figs 6.5&6.6 the traces were overdrawn, because of poor quality of the original printout).

2.10 Computing and statistical analyses

In order to determined if there was a signification difference between the plants under treatment and control, statistical analysis was used. ANOVA and TWO-WAY analysis of variance was used. F tables were used to find the significance of the results. The analysis of variance of (ANOVA) were performed by using Minitab soft-ware package through the Novel system of the Computer Center, University of Durham. The graphics were produced using Cricket graph software version 1.3.1 package in Word for Windows, version 3.1. All the routine calculations were performed on Microsoft Excel. The text was written up using Word for Windows version 6.00

2.11 Chemicals

All chemicals used were purchased from either BDH, Poole, or Sigma.

CHAPTER 3

3.1 Effect of water availability on stomatal patterns of pot-grown seedlings.

Preliminary experiments were performed to investigate the effect of limited water availability on the patterns of stomata in leaves of both wheat and sorghum, and to explore the magnitude of any response. It was also necessary to investigate the universality of any response within plant species. For this reason two contrasting grain plants were used: wheat having a mesic growth response and sorghum which is more tolerant to extremes of water availability.

In order to investigate the response batches of seedlings were exposed to three watering regimes; water given every day, every 4-days and every 7-days as detailed in Material and Methods (2.3.1).

Successive leaves produced, up to the 4th leaf, were used for growth records, and epidermal cell pattern determinations. Both blade and sheath were investigated as detailed in Material and Methods. Results were analysed as out-put from two-way ANOVA.

3.2 Wheat

3.2.1 Effects of watering regimes on growth

Seedlings of wheat grown under the different water regime conditions showed marked differences in their growth patterns. Plants watered at 4- or 7-day

intervals had reduced leaf growth when compared with the controls which were watered every day, Plate 3.1. These results show clearly that leaf growth was influenced by watering and that leaf cell patterns could be altered also.

Plate   3.1. Wheat plants grown for 20 days in soil where water was given every day (left), every 4 day (center) and every 7 days (right).

3.2.2 Effects of watering regimes on epidermal cell patterns.

Epidermal acetate impressions were used to determine overall cell patterns on the adaxial surface of the leaves. The impressions were made from the tip and basal regions of the blade, and from the mid region of the sheath from successive leaves. The adaxial and abaxial epidermes of wheat leaves had markedly uniform cells, stomata could be identified as pores surrounded by subsidiary cells and by the presence of two guard cells. Such stomata were found along the leaf. Plate 3.2 & 3.3. The cell patterns were determind for the adaxial surfaces only since more stomata were observed here.

Plate   3.2. Surface view of a nail varnish peel of the abaxial surface of a wheat.
S: Stoma
T; Trichome
Black bar = 50μm
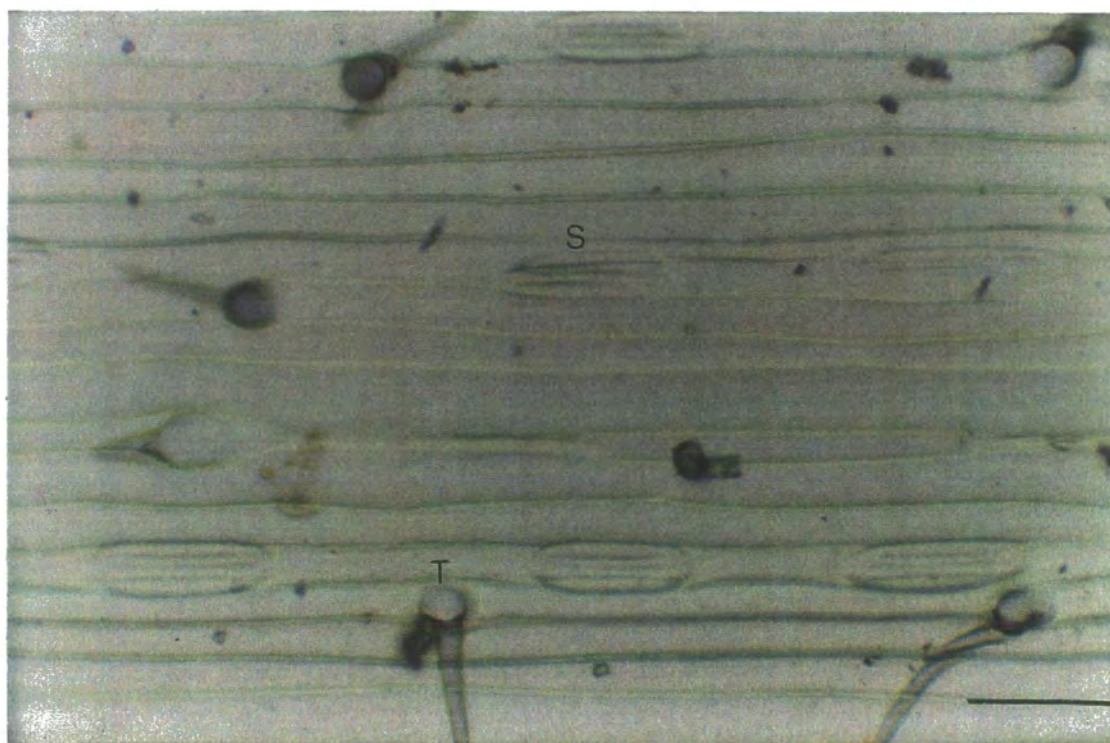
Plate 3.3. Transverse section through a wheat leaf showing the presence of stomata on the abaxial surface
Tissue was stained with toluidine blue.
S = Stoma
AS = Substomatal air space.
Black bar = 50µm
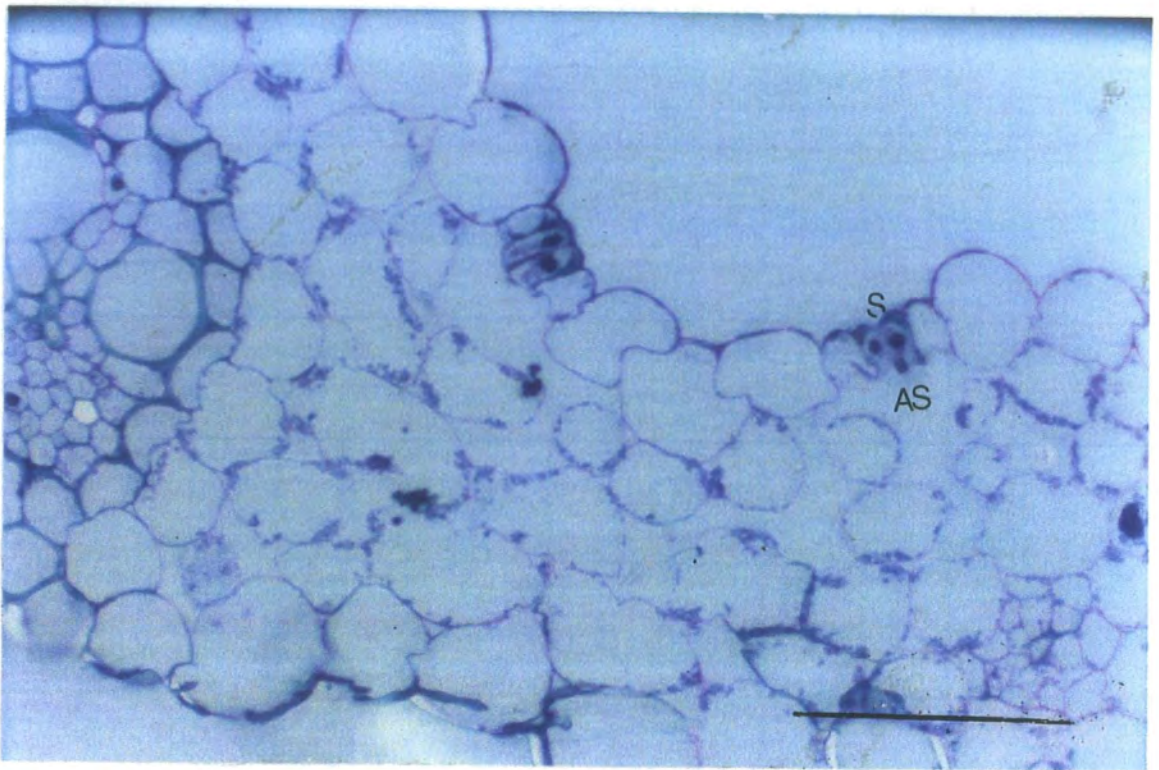
3.2.2.1 Epidermal cell density

The results of epidermal cell counts are shown in Tables 3.1, 3.2, 3.3 & 3.4 for successive leaves. It can be seen that significant differences exist between epidermal cell densities in all three portions of the leaves, tip, middle and base. With reduced frequency of watering the densities of epidermal cells increased. This effect was probably related to reduced water uptake resulting in smaller epidermal cells. All four leaves on each plant showed the same pattern of response but no consistent trend was noted for the patterns with leaf insertion point and leaf age, i.e. greater / lesser effect on older leaves. A similar response to water limitation was noted for the sheath tissues of all leaves.

3.2.2.2 Stomatal density

Similarly, the densities of stomata also changed with the watering regimes, increasing with the lower frequency of watering, Tables 3.1, 3.2, 3.3, & 3.4. This pattern can also be related to the effects of reduced leaf expansion. The increase in density of stomata, however, was not to the same extent as the increase in density of epidermal cells. In the plants watered every day and at 4-day intervals it was noted that the blades of all leaves had higher stomatal densities than the sheaths. However, this patterns was not noted in those plants watered at 7-day intervals.

Table 3.1. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²) and stomatal density (SD) (no/mm) of the blade tip and base regions, and sheath mid-region of leaf 1 of wheat (±SE).

Watering regimes	TIP		BASE		SHEATH	
	E.C.D	SD	E.C.D.	SD	E.C.D	SD.
Every day	207.04±17.89	23.66±6.18	215.86±12.74	39.65±2.70	383.26±9.68	33.04±3.87
Every4days	356.83±14.04	30.84±4.92	222.47±14.11	41.85±2.20	702.64±12.51	35.24±2.20
Every7days	759.91±29.64	36.38±3.34	414.09±20.19	45.03±3.11	911.89±8.34	39.32±2.11
F (df)	66.90 (2)	4.66 (2)	35.00 (2)	1.00 (2)	70.00 (2)	1.72 (2)
P≥	0.001	0.05	0.001	ns	0.001	ns

Table 3.2. Effects of watering regimes on the, epidermal cell density (ECD) (no/mm²) and stomatal density (SD) (no/mm²) of the blade tip and base regions, and sheath mid-region of leaf 2 of wheat (±SE).

Watering regimes	TIP		BASE		SHEATH	
	E.C.D.	SD.	E.C.D.	SD	E.C.D.	SD
Every day	240.09±10.99	28.64±2.69	204.84±11.91	30.84±2.20	387.66±10.91	26.43±2.69
Every4days	398.68±22.41	33.04±1.52	235.68±21.07	35.24±1.82	605.73±6.35	30.84±1.30
Every7days	816.47±12.84	35.24±2.21	522.03±21.35	37.44±2.39	907.49±7.47	37.44±2.88
F (df)	93.31 (2)	4.65 (2)	67.92 (2)	1.55 (2)	55.77 (2)	4 62(2)
P≥	0.001	0.05	0.001	ns	0.001	0.05

Table 3.3. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²) and stomatal density (SD) (no/mm²) of the blade tip and base regions, and sheath mid-region of leaf 3 of wheat (±SE).

Watering regimes	TIP		BASE		SHEATH	
	E.C.D.	SD	E.C.D.	SD	E.C.D.	SD
Every day	339.21±16.40	25.44±3.66	334.80±.9.63	33.04±0.01	370.04±15.72	25.42±1.39
Every 4days	671.81±6.11	37.44±2.00	431.71±6.63	37.45±1.60	486.78±13.70	29.83±1.47
Every 7days	784.14±11.25	39.65±2.00	599.12±5.91	44.05±1.39	852.42±22.43	36.43±2.45
F (df)	18.83 (2)	4.49 (2)	35.41 (2)	12.68 (2)	34.64 (2)	9.74 (2)
P≥	0.01	0.05	0.001	0.01	0.001	0.01

Table 3.4. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²) and stomatal density (SD) (no/mm²) of the blade tip and base regions, and sheath mid-region of leaf 4 of wheat (±SE).

Watering regimes	TIP		BASE		SHEATH	
	E.C.D.	SD	E.C.D.	SD	E.C.D.	SD
Every day	466.96±7.99	33.04±1.00	348.01±20.14	30.84±3.36	389.87±20.58	26.43±1.47
Every4days	740.09±4.98	35.24±1.38	546.26±30.36	39.65±5.14	505.33±39.04	30.84±1.73
Every7days	910.00±9.94	36.44±1.44	713.66±17.34	40.01±1.47	918.50±20.94	35.24±1.44
F (df)	47.94 (2)	1.00 (2)	46.97 (2)	9.35 (2)	45.17 (2)	4.66 (2)
P≥	0.001	ns	0.001	0.01	0.001	0.05

3.2.2.3 Stomatal index

When stomatal index was calculated it was noted that this parameter decreased with decreases in watering frequency in all leaves and in all portions of each leaf, Figs 3.1a, 3.2a & 3.3a, These results indicated an effect of water availability on the development of stomata independent of leaf expansion.

Whilst the patterns of stomatal index reduction were consistent in all leaves it can be seen that there were marked differences in stomatal indices between blades of successive leaves of control plants. These differences, however, were not so apparent in the plants with lowest water availability indicating that there were limitations to developmental changes possible. A lower limit of production of stomata may be operating in order to maintain some gaseous exchange. This pattern, however, was not so apparent in the sheath tissues. The comparison was made between all the four leaves under each individual treatment.
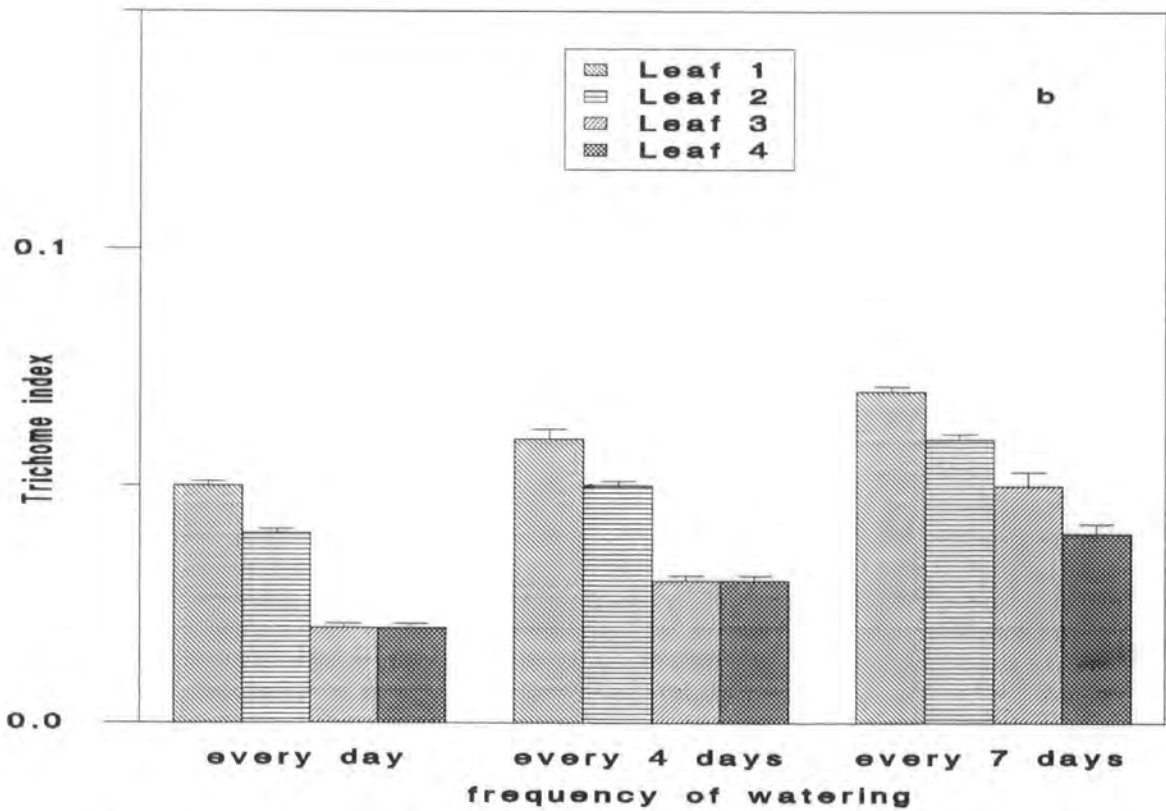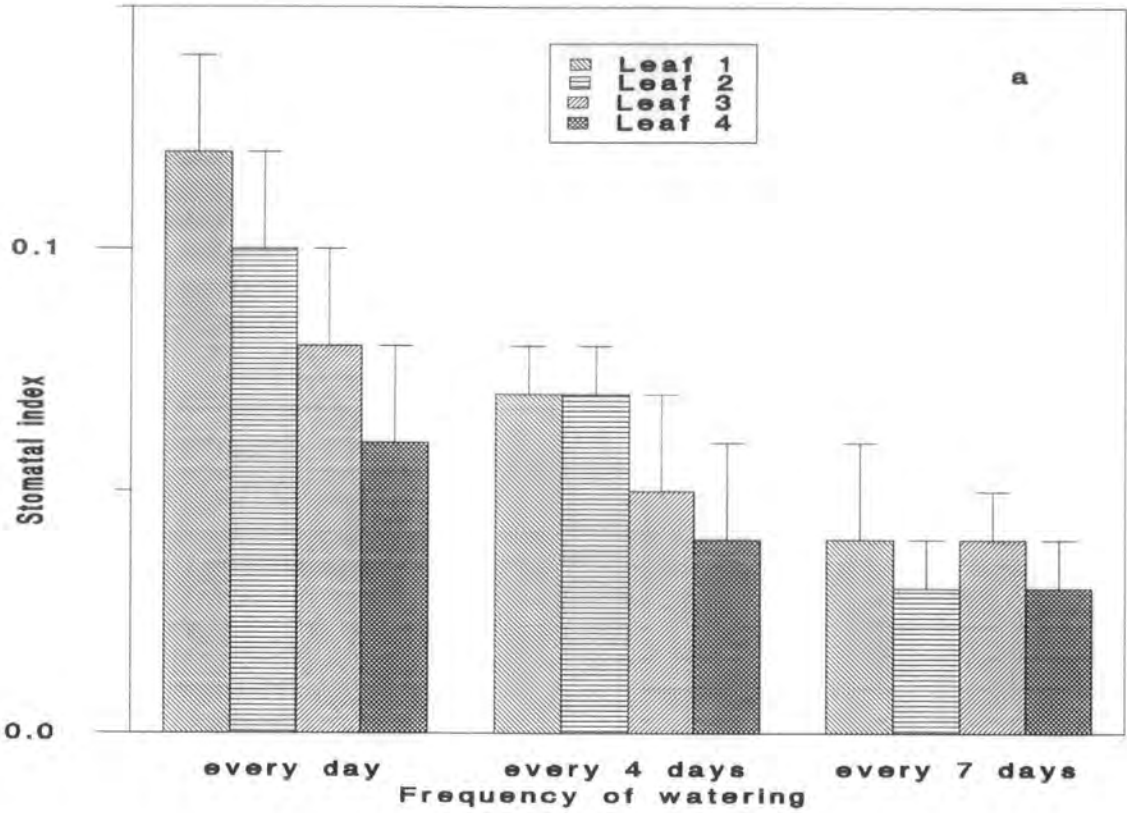
Fig. 3-1 Effects of different watering regimes on the stomatal index (a) and trichome index (b) of wheat seedlings, tip region of leaf blade.
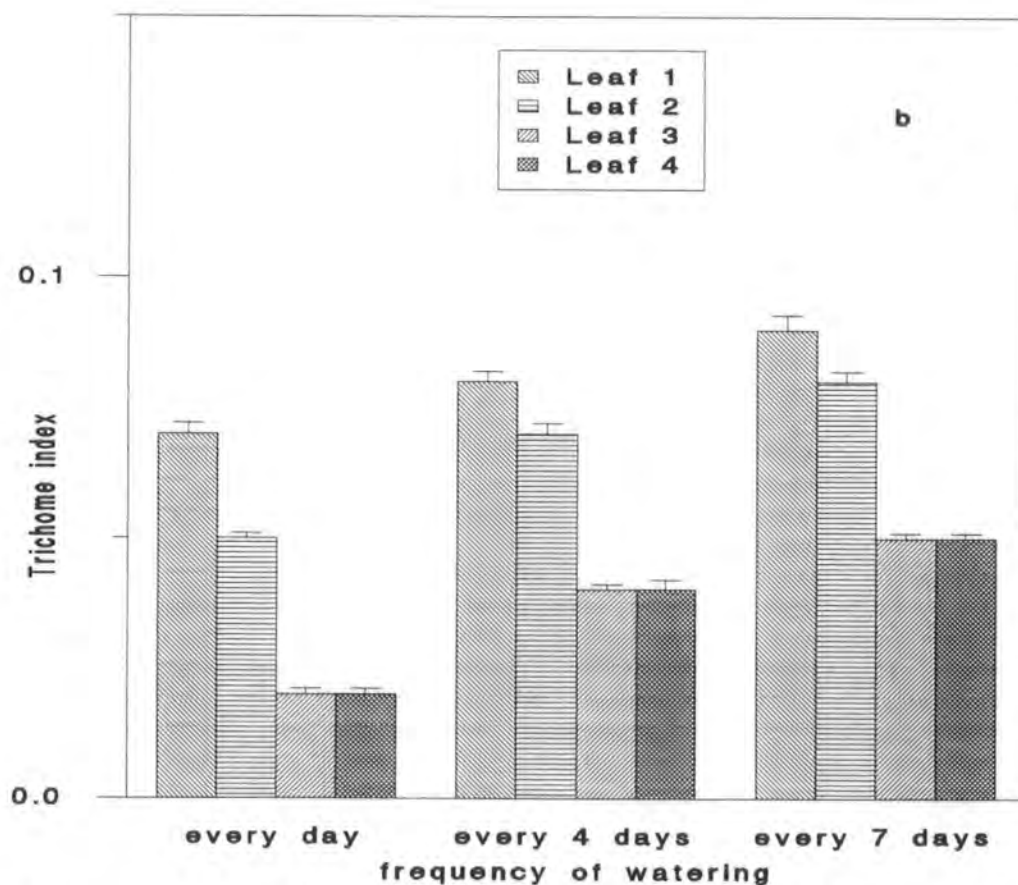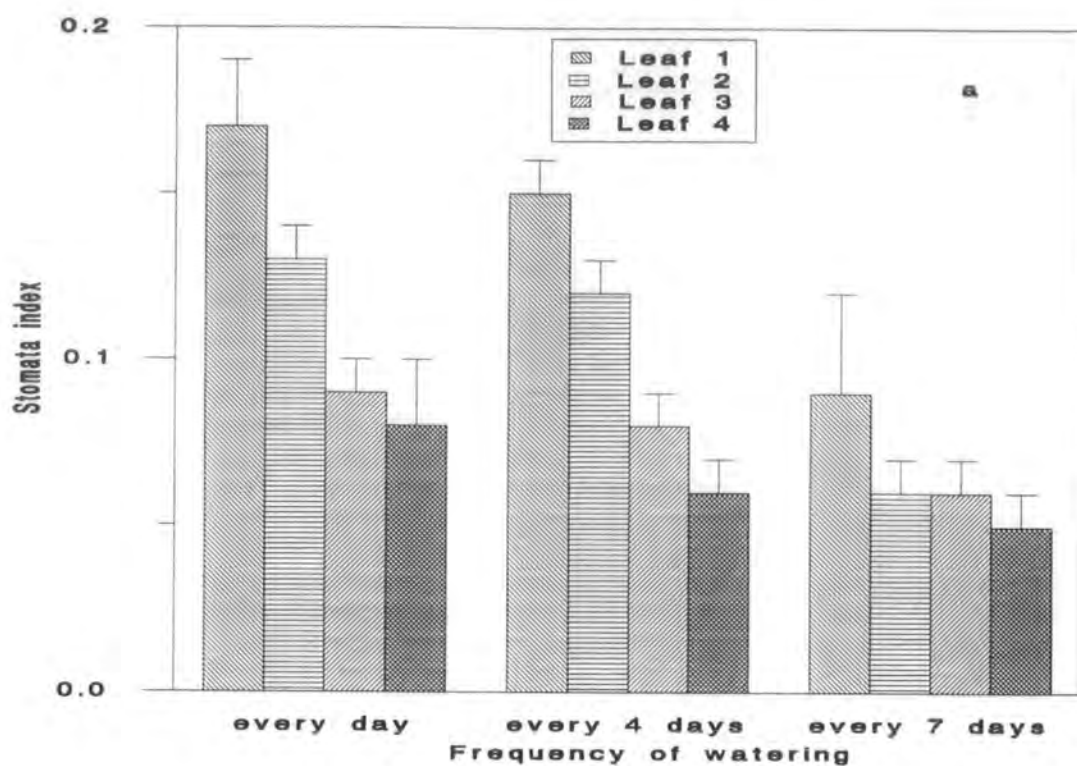
Fig. 3-2   Effects of different watering regimes on the stomatal index (a) and trichome index of wheat seedlings, base region of leaf blade.
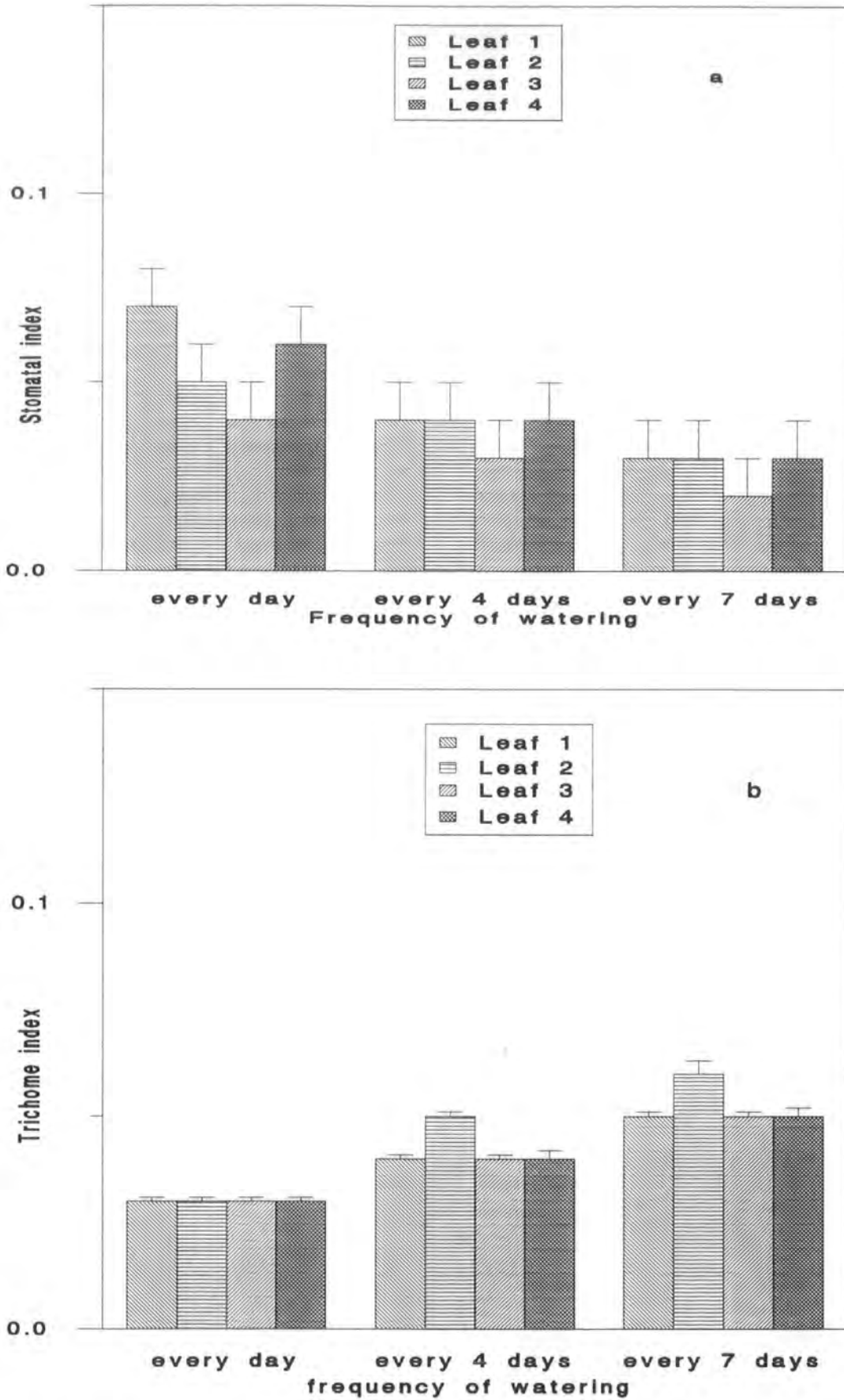
Fig. 3-3 Effects of different watering regimes on the stomatal index (a) and trichome index (b) of wheat seedlings, sheath mid- region.

3.2.2.4 Trichome index

It was noted that whilst there was a change in stomatal densities with watering regimes there was also a change in the apparent densities of trichomes. Plate 3.4 shows an apparent increase in the density of trichomes on the leaf surface following water limitation. The possibility of a relationship between these two components was examined using the calculation of the trichome index.

Decreases in stomatal index and increases in the trichome index are related directly to developmental switches in response to water limitation. Fig. 3.1b shows that the highest trichome index (0.06) was noted for the tip region of leaf 1 of plants grown under the 7-day watering regime while the lowest index (0.02) was observed with every-day watering. This response was consistent for all four leaves. There appears to be a reciprocal relationship between trichome index and stomatal index.

Plate 3.4. Nail varnish impressions of the abaxial surface of wheat leaves.

a. Plants watered every day. (S; Stoma T: Trichome Black bar = 50μm)
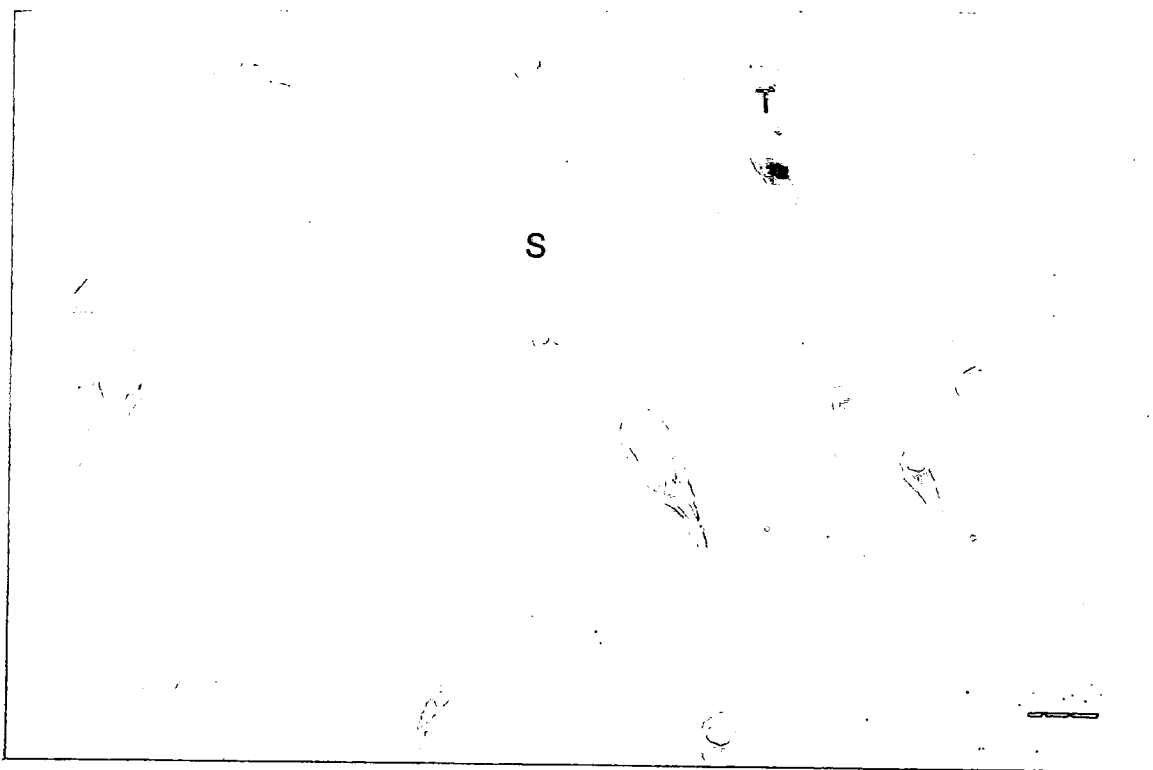
b. Plants watered every 7 days.
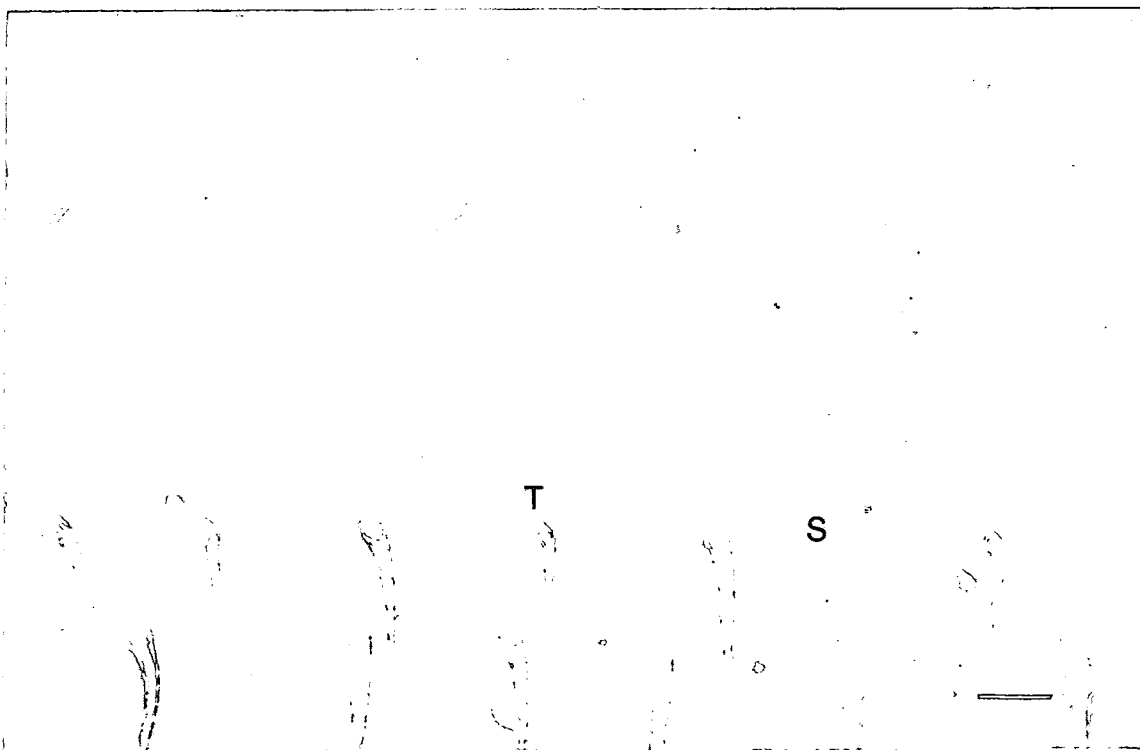
3.3 Sorghum

3.3.1 Effect of watering regimes on growth

For the purpose of comparison, six sorghum cultivars were grown under different water regime conditions, watered every day, every 4-days and every 7-days as for wheat. The different cultivars showed normal growth patterns when the treatment plants were compared with the controls, (watered every day). This species, and its cultivars were found to be much less sensitive to water stress then wheat plants in terms of their growth responses. Plate (3.5). Very little effect of water limitation on extension growth was noted.

These results indicate that plants growing in dry habitats posses a more efficient absorbing system relative to their transpiring surface and maintain a positive water balance which has limited effect on growth.

Plate 3.5. Sorghum plants grown for 20 days in soil where water was given every day (left), every 4 day (center) and every 7 days (right).

3.3.2 Effect of watering regimes on leaf epidermal cell patterns

3.3.2.1 Epidermal cell density

The imprint of abaxial surfaces of the leaves of each cultivar were used to determine the epidermal cell patterns. The stomatal counts were taken from the equivalent leaf regions used for wheat plants. The general trend in all cultivars was that epidermal cell density increased with decreased water availability in all three portions of the leaves,. Table 3.5-Table 3.28. A comparison of the different cultivars studied showed that there was a marked variation in absolute epidermal cell density, amongst them. Also density variation was noted within and between leaves of individual cultivars.

Table 3.5. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 1 of Saudi Red sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD.	SD	TI
Every day	127.75±1.05	33.04±3.67	0.08±0.03	154.18±6.81	28.64±1.30	0.06±0.01	151.98±4.01	22.03±5.52	0.04±0.01
Every 4 days	156.38±2.00	44.05±4.66	0.15±0.01	218.05±5.36	44.05±1.39	0.08±0.02	211.44±6.03	33.04±3.18	0.08±0.02
Every 7 days	176.21±2.62	52.89±1.69	0.17±0.03	244.66±6.49	48.45±1.47	0.11±0.01	279.72±8.90	44.05±2.00	0.11±0.03
F (df)	30.12 (2)	61.08 (2)	33.13 (2)	29.63 (2)	33.42 (2)	17.48 (2)	25.60 (2)	28.61 (2)	55.60 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.001

Table 3.6. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 2 of Saudi Red sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD.	SD	TI	ECD.	SD	TI	ECD.	SD	TI
Every day	167.39±5.81	33.04±1.18	0.06±0.01	178.94±8.64	22.03±2.47	0.09±0.01	167.40±3.36	20.13±2.02	0.03±0.01
Every 4 days	171.80±2.94	44.05±2.00	0.13±0.02	185.01±6.12	37.44±1.39	0.12±0.02	218.06±5.73	30.11±1.30	0.08±0.02
Every 7 days	181.01±4.36	52.86±3.62	0.17±0.03	197.24±7.56	46.25±1.47	0.15±0.02	264.31±7.52	46.25±1.44	0.09±0.01
F (df)	6.44 (2)	30.95 (2)	18.93 (2)	16.46 (2)	31.06 (2)	29.65 (2)	25.05 (2)	21.04 (2)	12.08 (2)
P≤	0.05	0.001	0.001	0.01	0.001	0.001	0.001	0.001	0.01

Table 3.7. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 3 of Saudi Red sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD.	SD	TI	ECD.	SD	TI	ECD	SD	TI
Every day	167.40±9.47	22.03±1.39	0.05±0.01	165.19±2.80	27.90±1.47	0.05±0.01	187.22±9.86	26.43±1.47	0.04±0.01
Every 4 days	178.40±5.96	35.24±2.01	0.11±0.02	196.02±4.59	41.11±2.00	0.10±0.03	244.48±5.39	39.65±1.39	0.07±0.02
Every 7 days	207.04±7.22	48.45±1.47	0.14±0.04	257.70±3.32	49.19±1.47	0.12±0.04	252.11±6.64	44.66±1.44	0.11±0.03
F (df)	29.37 (2)	31.90 (2)	19.52 (2)	29.71 (2)	38.29(2)	18.07 (2)	25.58 (2)	88.62 (2)	69.96 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.001

Table 3.8. Effects of watering regimes on the epidermal celldensity (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 4 of Saudi Red sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD.	SD	TI	ECD.	SD	TI	ECD	SD	TI
Every day	189.22±6.87	30.84±1.18	0.03±0.01	198.23±8.41	28.64±1.39	0.07±0.03	220.26±2.27	23.43±1.66	0.03±0.01
Every 4 days	222.46±7.86	14.85±3.30	0.11±0.02	226.87±3.85	39.65±1.18	0.09±0.01	237.88±6.58	33.04±1.68	0.07±0.02
Every 7 days	233.48±6.90	57.27±2.06	0.15±0.03	237.88±7.17	57.27±1.30	0.14±0.03	244.50±4.49	44.05±1.18	0.10±0.02
F (df)	27.35 (2)	16.14 (2)	64.03 (2)	22.09 (2)	36.63 (2)	68.93 (2)	19.12 (2)	49.47 (2)	25.55 (2)
P≤	0.001	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.9. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 1 of Funk sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	145.37±7.76	28.63±1.42	0.06±0.01	154.91±5.21	30.21±2.33	0.10±0.01	171.06±6.35	20.56±1.00	0.03±0.01
Every 4 days	201.90±6.14	39.64±1.44	0.11±0.03	208.51±5.42	48.46±1.30	0.12±0.04	265.04±12.42	33.04±1.68	0.08±0.03
Every 7 days	258.43±7.28	50.66±2.20	0.13±0.01	265.77±4.14	61.67±1.41	0.13±0.03	328.32±6.68	41.85±1.18	0.09±0.02
F (df)	22.92 (2)	19.83 (2)	26.64 (2)	30.24 (2)	46.07 (2)	24.86 (2)	81.25 (2)	81.30 (2)	15.41 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01

Table 3.10. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions and mid-sheath region of leaf 2 of Funk sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	E.C.D.	SD	TI	E.C.D.	SD	TI	E.C.D.	SD	TI
Every day	97.65±4.14	23.49±2.11	0.06±0.01	118.20±3.13	35.24±1.39	0.08±0.01	158.58±11.46	17.62±1.39	0.04±0.01
Every 4 days	126.21±5.35	30.83±1.18	0.12±0.03	143.17±3.87	42.58±1.00	0.15±0.03	210.71±2.60	24.23±1.18	0.06±0.02
Every 7 days	169.60±3.36	40.55±1.44	0.16±0.02	197.49±2.22	59.19±2.22	0.17±0.03	268.71±4.90	31.57±1.00	0.07±0.02
F (df)	97.99 (2)	34.26 (2)	29.80 (2)	47.69 (2)	86.03 (2)	23.66 (2)	55.11 (2)	31.89 (2)	7.49 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.05

Table 3.11. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 3 of Funk sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	124.82±4.77	29.37±1.39	0.08±0.01	116.00±4.54	38.91±1.47	0.13±0.01	195.29±3.79	22.76±2.84	0.04±0.01
Every 4 days	145.37±5.50	41.11±1.30	0.15±0.03	147.57±5.46	50.66±1.44	0.16±0.04	241.55±6.03	24.11±1.47	0.10±0.02
Every 7 days	185.01±6.38	55.06±2.15	0.17±0.02	186.48±6.49	60.94±1.47	0.17±0.03	311.29±12.55	36.71±2.32	0.12±0.03
F (df)	27.38 (2)	68.24 (2)	23.34 (2)	36.56 (2)	63.48 (2)	12.07 (2)	40.90 (2)	18.69 (2)	27.38 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.001

Table 3.12. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 4 of Funk sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	105.72±2.35	21.60±1.44	0.09±0.01	113.06±4.09	36.71±1.38	0.10±0.01	154.91±8.49	16.15±1.47	0.05±0.01
Every 4 days	132.89±3.13	37.44±1.30	0.16±0.03	130.68±2.06	41.85±1.59	0.17±0.03	219.52±3.79	23.49±1.00	0.10±0.02
Every 7 days	149.77±3.69	41.01±1.86	0.18±0.01	160.79±3.36	52.13±1.30	0.19±0.01	247.42±3.01	27.16±1.47	0.11±0.01
F (df)	96.89 (2)	35.33 (2)	16.70 (2)	88.80 (2)	31.55 (2)	25.95 (2)	32.12 (2)	17.53 (2)	33.04 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.13. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 1 of Indian White sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	140.23±6.58	23.49±1.00	0.05±0.01	166.66±2.82	35.97±1.39	0.08±0.02	152.71±3.70	20.56±1.00	0.03±0.01
Every 4 days	171.06±7.20	29.37±1.39	0.09±0.01	190.15±2.73	42.58±1.30	0.12±0.03	193.63±6.78	26.33±5.58	0.11±0.02
Every 7 days	202.63±5.35	33.11±1.39	0.14±0.03	223.19±8.63	52.13±2.00	0.14±0.03	193.82±6.78	38.18±1.47	0.12±0.03
F (df)	50.25 (2)	26.09 (2)	26.57 (2)	93.25 (2)	31.03 (2)	30.02 (2)	86.27 (2)	36.35 (2)	34.36 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.14. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions and sheath mid-region of leaf 2 of Indian White sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	165.92±1.69	31.57±3.87	0.08±0.01	143.90±2.73	32.30±2.84	0.09±0.01	144.63±4.79	24.79±1.18	0.02±0.01
Every 4days	196.76±3.20	40.24±1.48	0.12±0.03	168.13±5.09	40.38±1.39	0.13±0.03	168.13±7.33	28.63±1.44	0.10±0.02
Every 7 days	237.87±4.27	48.46±1.44	0.14±0.02	196.76±8.59	47.91±2.27	0.16±0.03	248.89±7.81	33.32±1.69	0.12±0.01
F (df)	49.86 (2)	56.61 (2)	18.88 (2)	89.27 (2)	20.18 (2)	27.73 (2)	85.17 (2)	40.17 (2)	25.59 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.15. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 3 of Indian White sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	132.15±1.86	33.04±1.00	0.06±0.01	124.81±4.77	37.44±1.46	0.07±0.01	149.04±5.97	25.90±1.46	0.05±0.01
Every 4 days	162.25±3.64	42.14±1.44	0.11±0.01	143.16±3.72	43.32±2.84	0.15±0.03	188.68±1.82	36.71±1.39	0.11±0.03
Every 7 days	197.49±3.64	50.66±1.43	0.16±0.02	180.61±2.09	49.92±1.47	0.17±0.02	226.86±6.69	45.52±1.81	0.13±0.02
F (df)	19.94 (2)	62.23 (2)	37.66 (2)	47.76 (2)	21.27 (2)	22.42 (2)	38.70 (2)	30.56 (2)	23.31 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.16. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 4 of Indian White sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	112.33±3.90	30.10±1.30	0.07±0.01	135.09±2.27	42.58±1.00	0.05±0.01	134.35±5.71	27.16±1.47	0.05±0.01
Every 4 days	148.30±4.14	39.64±1.44	0.15±0.03	155.65±4.14	48.45±1.44	0.12±0.01	268.55±4.34	36.71±1.39	0.09±0.01
Every 7 days	168.86±1.39	40.22±1.47	0.18±0.02	184.28±2.51	57.27±1.59	0.18±0.02	260.63±8.37	47.72±1.38	0.14±0.03
F (df)	78.23 (2)	48.50 (2)	28.24 (2)	20.82 (2)	34.25 (2)	29.03 (2)	32.85 (2)	44.86 (2)	27.94 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.17. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 1 of Morgon 854 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD.	SD	TI	ECD.	SD	TI
Every day	142.43±8.63	19.82±1.18	0.03±0.01	156.99±5.39	26.43±1.30	0.05±0.01	165.92±6.40	20.22±1.18	0.03±0.01
Every 4 days	174.00±4.05	24.96±1.66	0.09±0.01	202.63±3.00	35.14±1.39	0.10±0.02	209.98±6.12	25.70±1.39	0.07±0.02
Every 7 days	219.25±3.79	37.44±1.69	0.11±0.03	228.33±7.33	45.52±2.37	0.14±0.02	287.07±2.73	30.17±1.30	0.09±0.03
F (df)	93.22 (2)	50.06 (2)	41.90 (2)	39.20 (2)	23.91 (2)	49.89 (2)	95.32 (2)	42.30 (2)	33.61 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.18. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 2 of Morgon 854 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	165.19±4.92	28.63±1.44	0.04±0.01	146.84.±2.56	33.04±1.47	0.08±0.01	168.13±3.64	24.23±1.18	0.04±0.01
Every 4 days	215.85±5.13	30.14±1.00	0.10±0.02	180.61±5.46	40.15±1.39	0.12±0.02	188.68±3.20	24.33±1.30	0.10±0.02
Every 7 days	269.44±4.91	49.92±1.47	0.11±0.01	220.99±5.73	51.39±1.47	0.15±0.02	257.70±6.34	31.61±1.46	0.12±0.02
F (df)	90.53 (2)	25.91 (2)	26.87 (2)	86.81 (2)	76.15 (2)	27.56 (2)	87.78(2)	43.19 (2)	30.65 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.19. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 3 of Morgon 854 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	145.37±1.17	30.84±1.18	0.08±0.01	140.96±2.20	38.91±1.47	0.10±0.03	142.43±5.53	21.16±1.30	0.06±0.01
Every 4 days	182.81±4.40	39.64±2.01	0.10±0.02	147.57±3.99	41.85±1.18	0.14±0.01	222.26±7.41	35.97±1.39	0.10±0.02
Every 7 days	221.72±9.05	49.19±1.47	0.14±0.03	181.34±4.54	50.66±1.48	0.18±0.02	254.76±7.43	40.10±2.00	0.13±0.03
F (df)	83.85 (2)	27.29 (2)	23.50 (2)	42.14 (2)	19.13(2)	32.28 (2)	97.60 (2)	47.42 (2)	26.99 (2)
P≤	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.001	0.001

Table 3.20. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 4 of Morgon 854 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	126.28±3.0	25.66±1.18	0.07±0.01	130.68±3.70	40.38±1.39	0.06±0.01	174.73±2.37	24.23±1.66	0.03±0.01
Every 4 days	167.39±5.1	39.64±1.41	0.09±0.01	160.05±3.20	52.13±1.30	0.13±0.04	243.01±3.48	35.24±1.68	0.06±0.02
Every 7 days	210.71±2.7	48.46±2.06	0.14±0.02	190.89±4.77	60.94±1.47	0.17±0.02	290.74±5.35	41.11±2.27	0.10±0.02
F (df)	28.98 (2)	44.97 (2)	18.54 (2)	35.49 (2)	14.86 (2)	42.57 (2)	18.56 (2)	49.42 (2)	31.99 (2)
P≤	0.001	0.001	0.001	0.001	0.01	0.001	0.01	0.001	0.001

Table 3.21. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 1 of Morgon 856 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	132.89±5.09	28.63±1.44	0.06±0.01	127.75±3.00	33.04±1.39	0.06±0.01	143.16±2.26	17.62±1.44	0.04±0.01
Every 4 days	174.00±3.90	32.14±1.44	0.10±0.02	157.11±2.94	41.11±1.30	0.13±0.04	198.96±2.27	26.43±1.68	0.07±0.02
Every 7 days	226.86±10.5	46.25±2.20	0.14±0.03	186.48±3.94	49.19±1.47	0.17±0.03	319.37±9.12	34.85±1.18	0.09±0.02
F (df)	38.11 (2)	20.56 (2)	20.36 (2)	28.81 (2)	30.39 (2)	20.89 (2)	83.79 (2)	69.83 (2)	16.39 (2)
P≤	0.001	0.01	0.01	0.001	0.001	0.01	0.001	0.001	0.01

Table 3.22. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 2 of Morgon 856 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	127.22±3.08	27.16±1.47	0.09±0.04	126.28±2.11	42.31±1.39	0.06±0.01	154.91±4.62	25.70±1.39	0.04±0.01
Every 4 days	158.11±3.76	32.14±1.44	0.14±0.03	145.37±4.32	47.72±1.38	0.15±0.04	191.62±2.35	30.83±1.18	0.10±0.03
Every 7 days	212.91±5.00	51.16±1.44	0.16±0.02	200.43±3.94	56.53±2.11	0.16±0.02	222.46±3.90	37.44±1.44	0.15±0.02
F (df)	52.16 (2)	56.32 (2)	20.94 (2)	26.35 (2)	20.31 (2)	18.37 (2)	85.34 (2)	21.26 (2)	23.94 (2)
P≤	0.001	0.001	0.01	0.001	0.01	0.001	0.001	0.01	0.01

Table 3.23. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 3 of Morgon 856 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	125.92±3.90	34.51±1.00	0.09±0.01	111.60±2.11	42.58±1.00	0.05±0.01	152.71±5.47	24.23±1.18	0.05±0.01
Every 4 days	159.32±2.11	48.46±1.44	0.14±0.03	137.29±2.60	52.56±1.44	0.11±0.04	182.81±3.99	28.63±1.68	0.10±0.01
Every 7 days	180.61±3.00	54.26±2.20	0.18±0.02	164.46±3.31	62.20±1.47	0.19±0.03	242.28±2.40	38.91±1.47	0.12±0.03
F (df)	93.72 (2)	39.92 (2)	26.24 (2)	91.12 (2)	39.83 (2)	34.86 (2)	45.63 (2)	26.95 (2)	25.36 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 3.24. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions and sheath mid-region of leaf 4 of Morgon 856 sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	100.58±1.82	33.04±1.44	0.09±0.01	110.13±3.22	41.85±1.18	0.10±0.01	107.92±2.20	20.12±1.18	0.05±0.01
Every 4 days	124.08±3.94	41.11±1.30	0.17±0.02	139.69±6.76	52.41±1.30	0.18±0.04	154.18±6.17	28.63±1.68	0.12±0.03
Every 7 days	158.58±2.35	50.66±1.44	0.20±0.03	168.13±1.30	61.67±1.47	0.20±0.02	209.98±6.92	40.38±1.39	0.15±0.02
F (df)	39.06 (2)	28.84 (2)	37.39 (2)	85.40 (2)	48.50 (2)	20.40 (2)	29.78 (2)	84.41 (2)	28.84 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

113

Table 3.25. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 1 of Pioneer 8ss sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	151.24±5.21	25.70±1.39	0.09±0.01	151.98±4.05	30.10±1.30	0.10±0.03	149.04±6.35	20.11±1.52	0.07±0.02
Every 4 days	179.87±4.25	30.28±1.23	0.10±0.02	186.62±1.95	38.91±1.47	0.13±0.04	255.39±2.11	27.16±1.47	0.08±0.02
Every 7 days	231.27±1.86	36.98±2.74	0.11±0.02	229.06±3.43	49.19±1.46	0.14±0.02	308.36±10.4	36.71±2.27	0.09±0.01
F (df)	89.93 (2)	32.45 (2)	1.51 (2)	24.59 (2)	39.12 (2)	8.80 (2)	32.63 (2)	33.36 (2)	14.77 (2)
P≤	0.001	0.001	ns	0.001	0.001	0.05	0.001	0.001	0.05

Table 3.26. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (SI) of the blade tip and base regions, and sheath mid- region of leaf 2 of Pioneer 8ss sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	143.90±5.83	30.10±1.30	0.11±0.03	143.90±2.27	40.30±1.39	0.14±0.02	167.39±2.20	22.03±1.12	0.04±0.01
Every 4 days	190.15±5.73	39.64±1.44	0.12±0.01	175.47±1.69	49.29±1.47	0.16±0.03	201.90±4.52	27.90±1.47	0.07±0.02
Every 7 days	231.27±3.01	51.39±1.39	0.13±0.02	208.51±2.73	59.47±1.44	0.17±0.02	280.46±6.07	33.14±1.44	0.09±0.01
F (df)	95.88 (2)	23.70 (2)	1.89 (2)	85.55 (2)	42.31 (2)	10.41 (2)	49.34(2)	28.21 (2)	12.32 (2)
P≤	0.001	0.001	ns	0.001	0.001	0.01	0.001	0.001	0.01

Table 3.27. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 3 of Pioneer 8ss sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	110.86±2.27	33.04±1.26	0.13±0.01	114.54±2.80	38.18±1.47	0.13±0.03	157.11±4.49	24.96±1.30	0.07±0.01
Every 4 days	160.79±5.96	41.46±2.01	0.14±0.04	147.57±3.36	48.26±2.00	0.14±0.02	184.28±3.31	29.37±1.39	0.10±0.03
Every 7 days	204.10±2.82	58.73±1.39	0.16±0.02	179.14±1.30	50.21±1.47	0.15±0.03	230.53±2.51	33.91±1.44	0.12±0.01
F (df)	38.61 (2)	56.74 (2)	4.69 (2)	32.36 (2)	57.66 (2)	1.64 (2)	44.12 (2)	21.42 (2)	15.34 (2)
P≤	0.001	0.001	0.05	0.001	0.001	ns	0.001	0.01	0.01

Table 3.28. Effects of watering regimes on the epidermal cell density (ECD) (no/mm²), stomatal density (SD) (no/mm²) and trichome index (TI) of the blade tip and base regions, and sheath mid-region of leaf 4 of Pioneer 8ss sorghum (±SE).

Watering regimes	TIP			BASE			SHEATH		
	ECD	SD	TI	ECD	SD	TI	ECD	SD	TI
Every day	116.00±3.70	36.71±1.39	0.03±0.01	108.66±3.37	41.11±1.39	0.15±0.04	143.90±4.74	22.02±1.86	0.07±0.01
Every 4 days	164.46±9.08	46.15±1.47	0.11±0.02	130.72±5.97	43.92±1.18	0.17±0.03	185.01±3.26	29.37±1.68	0.08±0.02
Every 7 days	221.72±3.85	61.67±1.44	0.15±0.03	162.99±2.20	63.14±1.30	0.19±0.02	264.31±9.49	34.11±1.30	0.10±0.02
F (df)	29.81 (2)	41.31 (2)	34.03 (2)	39.16 (2)	52.99 (2)	18.82 (2)	83.19 (2)	24.51 (2)	15.41 (2)
P≤	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.01

3.3.2.2 Stomatal density

Sorghum varieties had marginally greater stomatal densities when compared with wheat. Stomatal density increased significantly (P = ≤ 0.001) in each leaf, in all 3 portions, and in each variety in response to reduced water availability.

Density in all cultivars was highest in the plants watered at 7-day intervals. the values for the plants watered every 4-days were lower than the 7-day watering intervals but higher than the control. This again indicates an effect on cell expansion.

3.3.2.3 Stomatal index

No consistent effect of water availability on stomatal indices was noted in the sorghum cultivars. Most cultivars, Funk, Indian White, & Morgon 854 showed no change in stomatal index under 4-day or 7-day watering regimes when compared with the controls, Figs. 3.5, 3.6 & 3.7. However, in two cultivars Morgon 856 & Pioneer 8ss a slight, non-significant decrease in stomatal index was noted under the 7-day watering regime, Figs. 3.8 & 3.9.

The Saudi Red cultivar showed a slight increase in the stomatal index in 4-day and 7-day watering regimes, Fig. 3.4. It can be seen that the index increases with age of leaves in the cultivars Funk, Indian White, Morgon 854, Morgon 856,

and Pioneer 8ss, Figs. 3.5-3.9 whereas in Saudi Red sorghum it decreases, Fig 3.4.

These changes in stomatal index are a reflection of leaf insertion and are consistent

in the control and treated plants.

In general, leaf cell patterns were less responsive in sorghum than in wheat

to limitation of water supply.
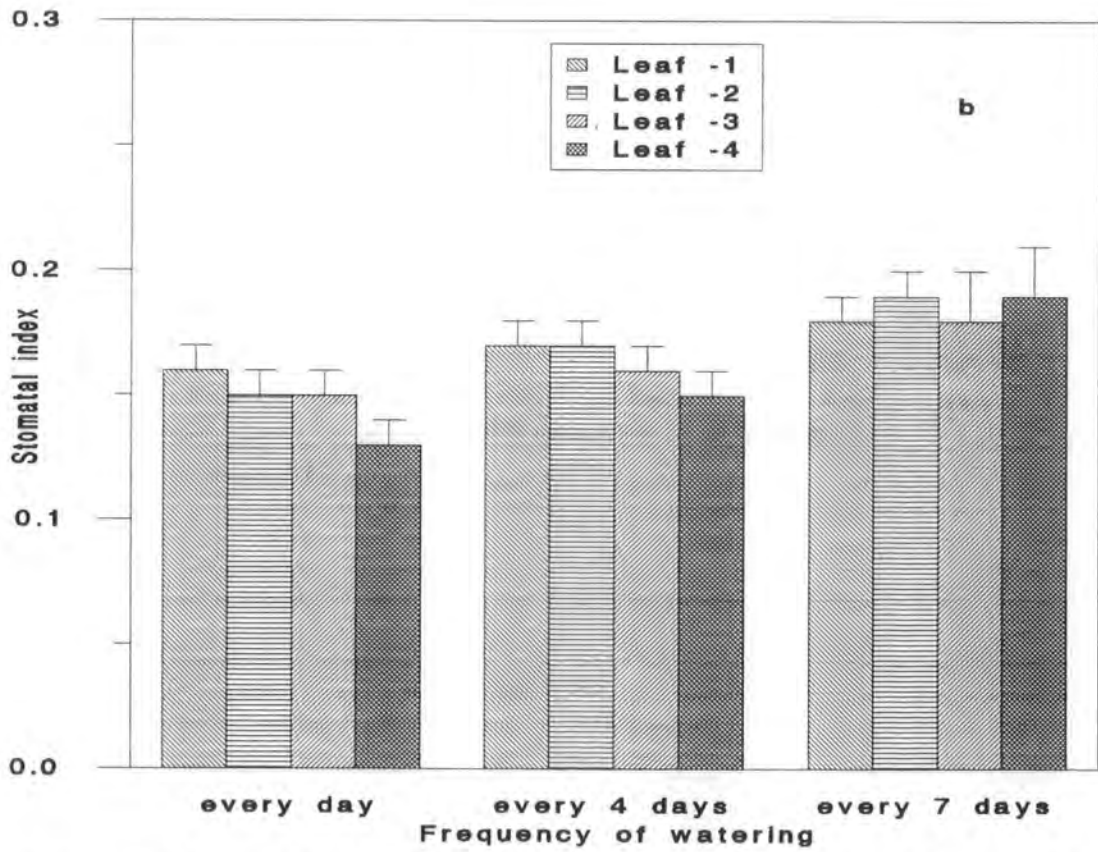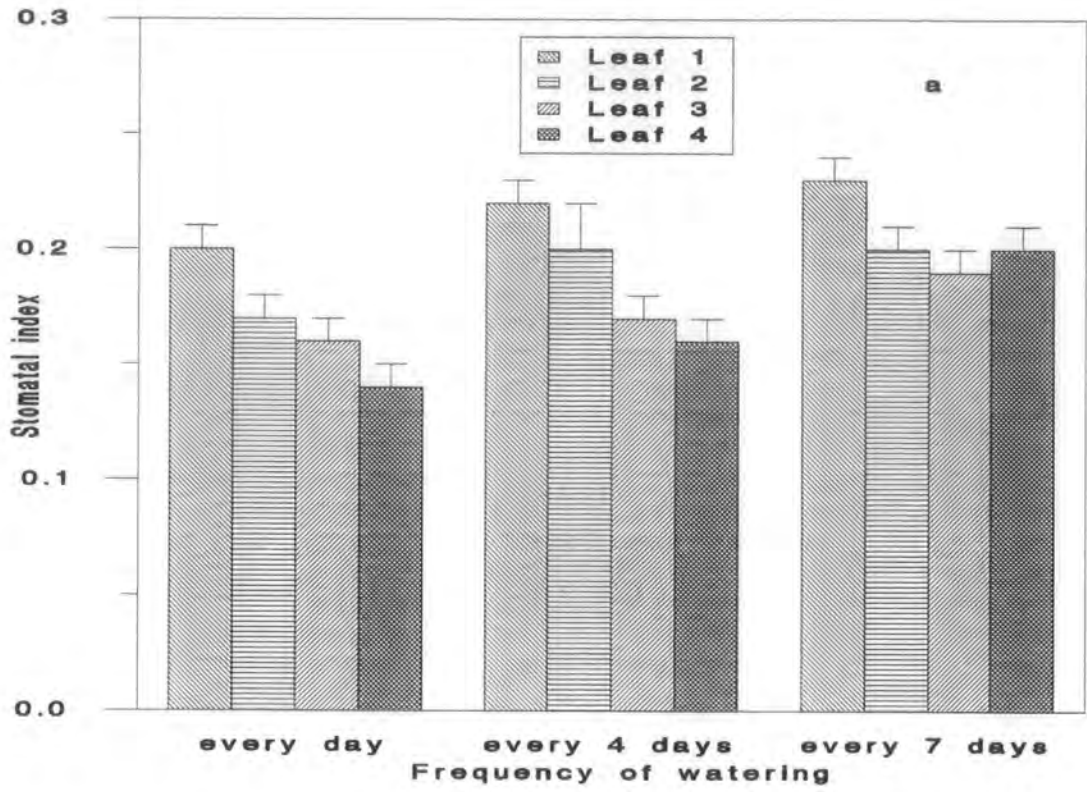
Fig. 3-4 Effects of different watering regimes on the stomatal index of Saudi Red sorghum seedlings, in the tip region (a), base region (b) and sheath mid-region (c-over).
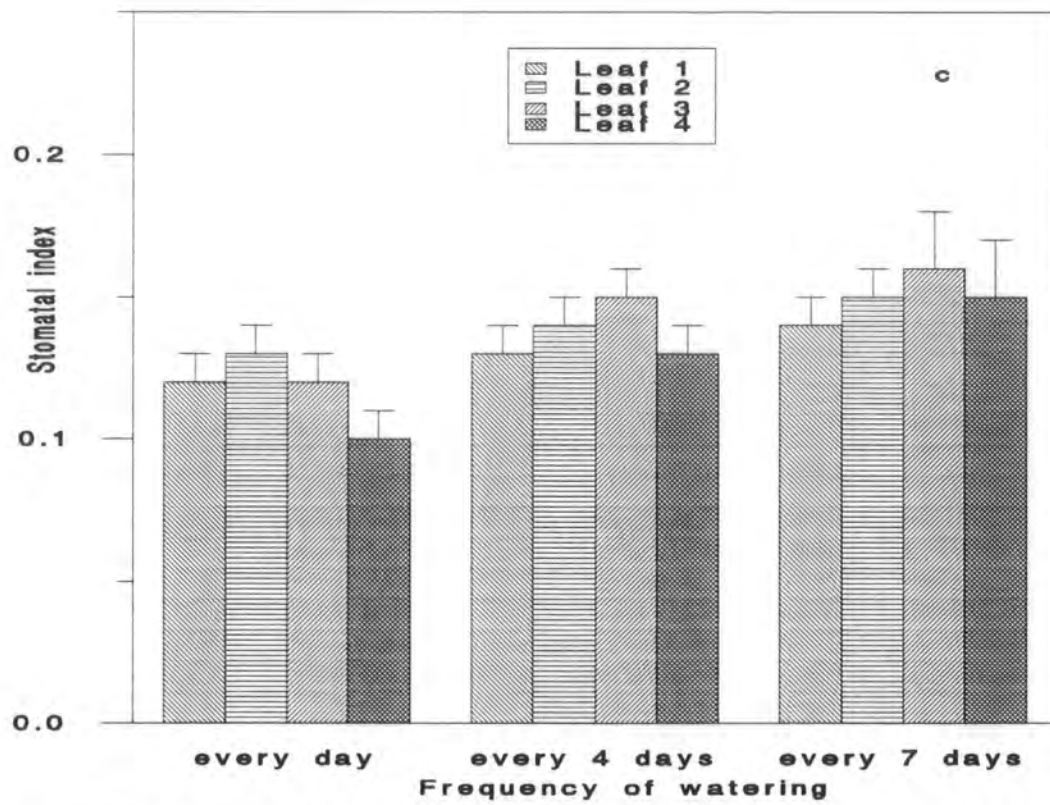
Fig. 3-4 cont.
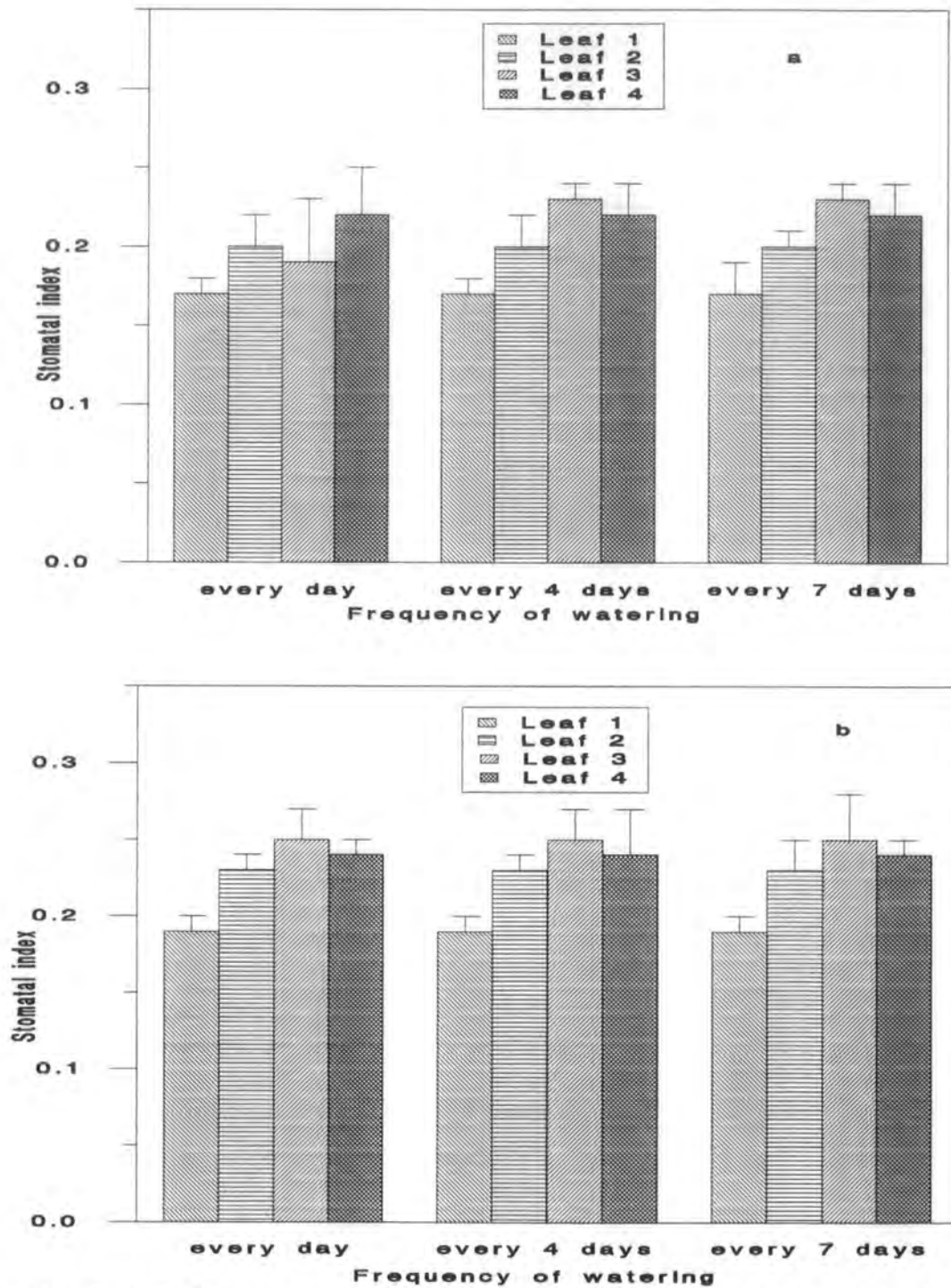
Fig 3-5 Effects of different watering regimes on the stomatal index of Funk sorghum seedlings, in the tip region (a), base region (b) and sheath mid-region (c-over).
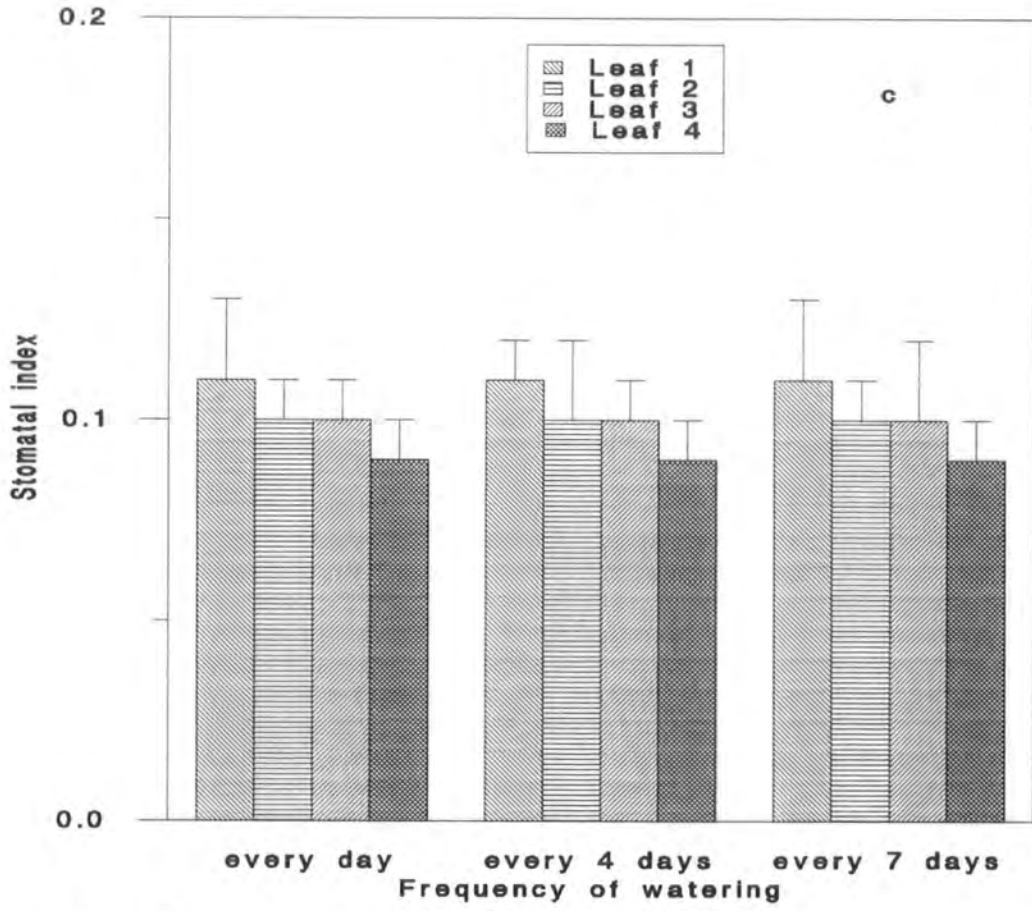
Fig. 3-5   cont.
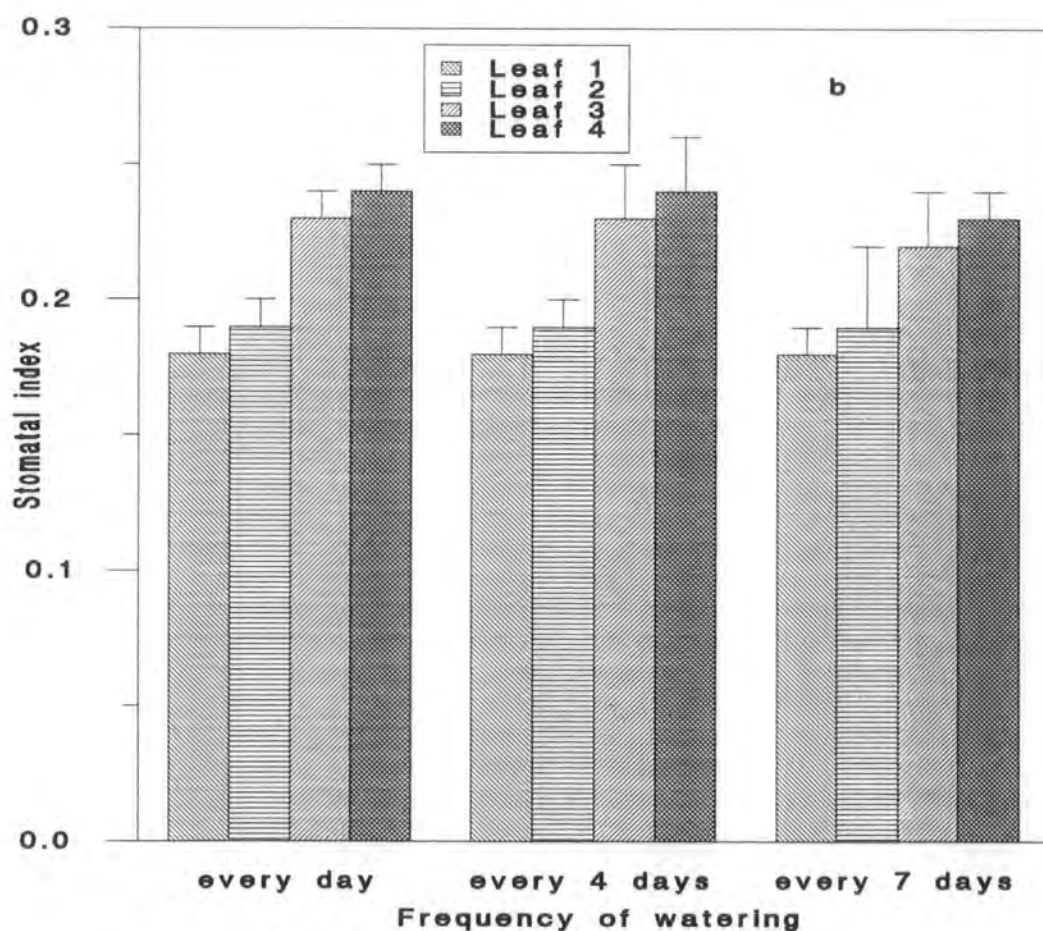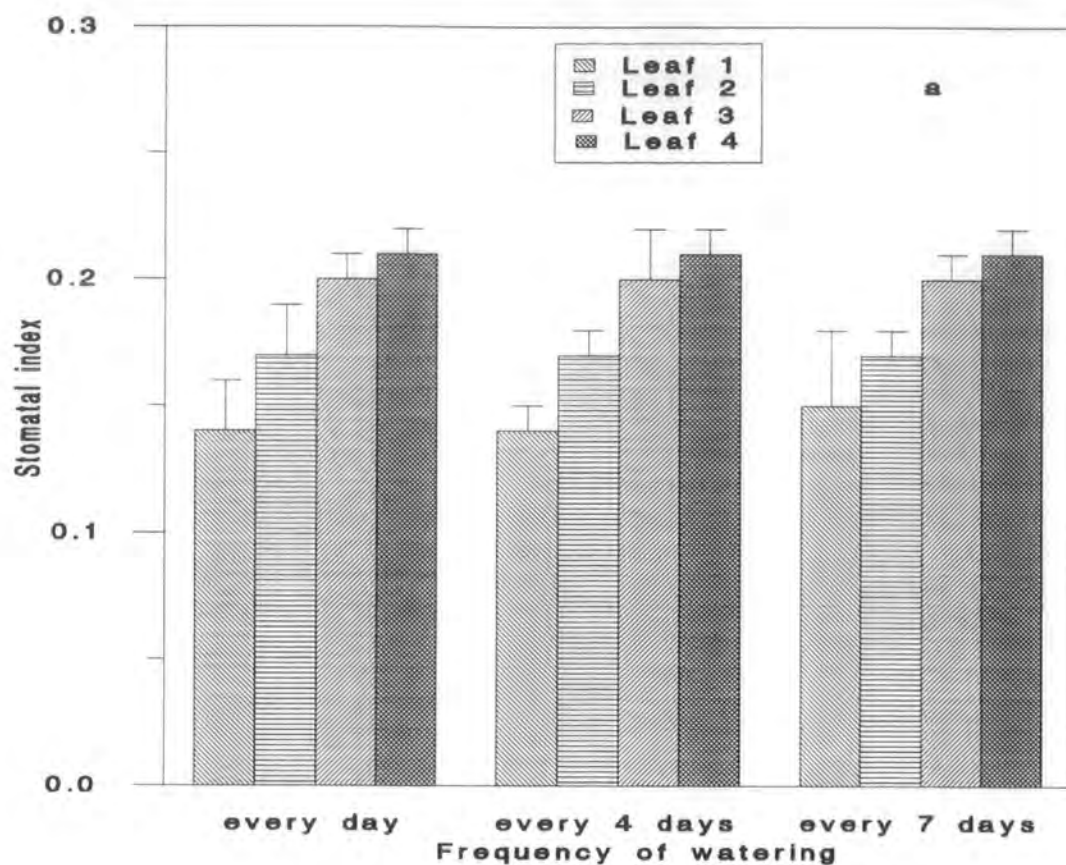
Fig. 3-6 Effects of different watering regimes on the stomatal index of Indian White sorghum seedlings, in the tip region (a), base region (b) and sheath mid-region (c-over).
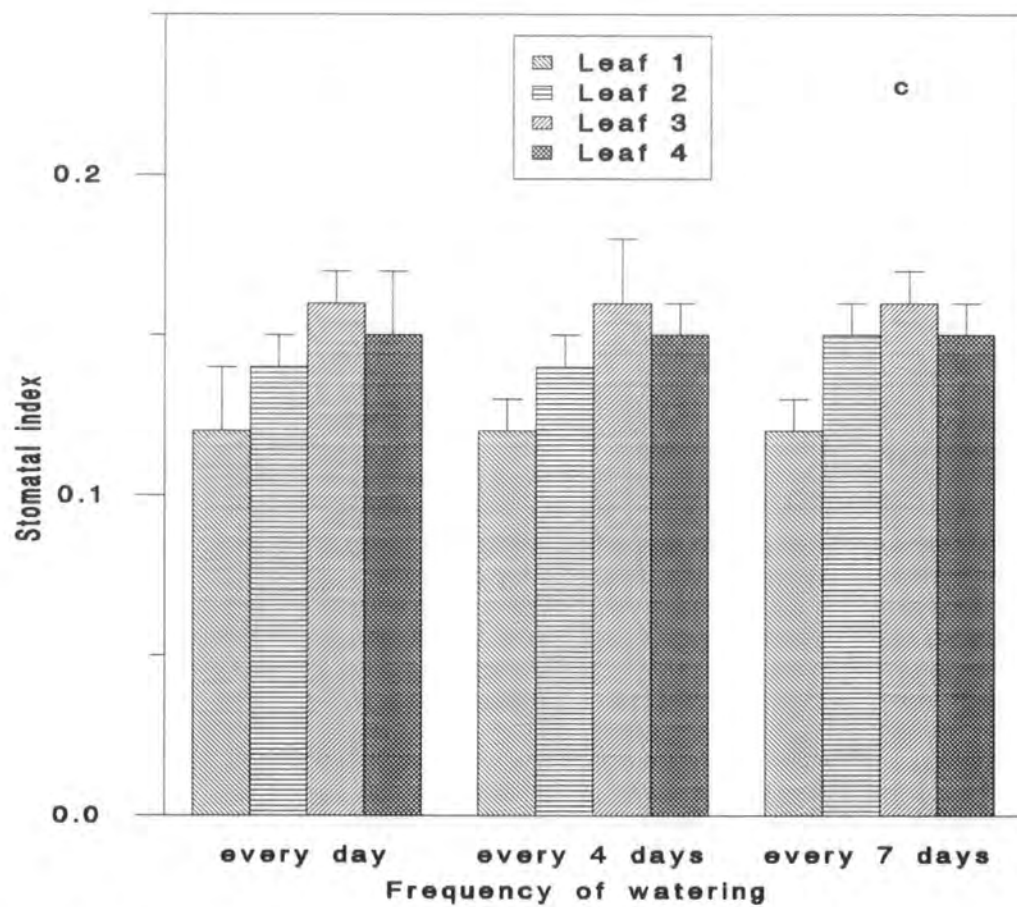
Fig 3-6   cont.
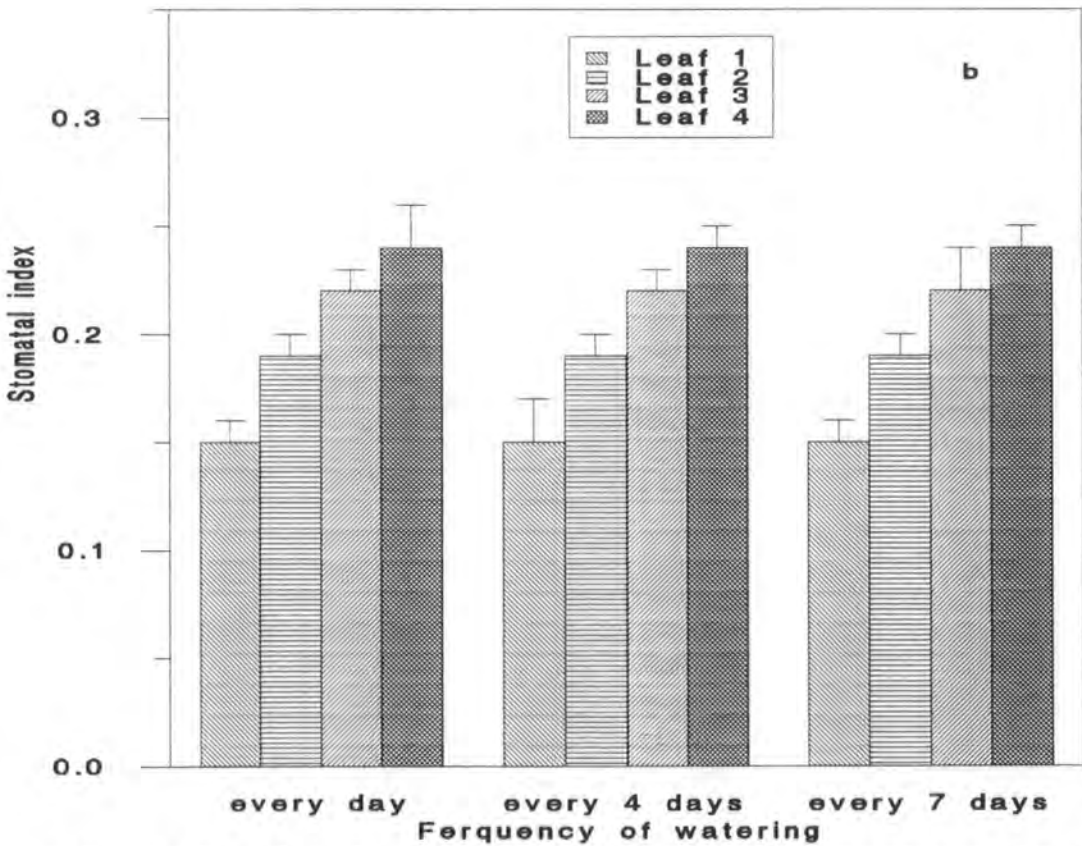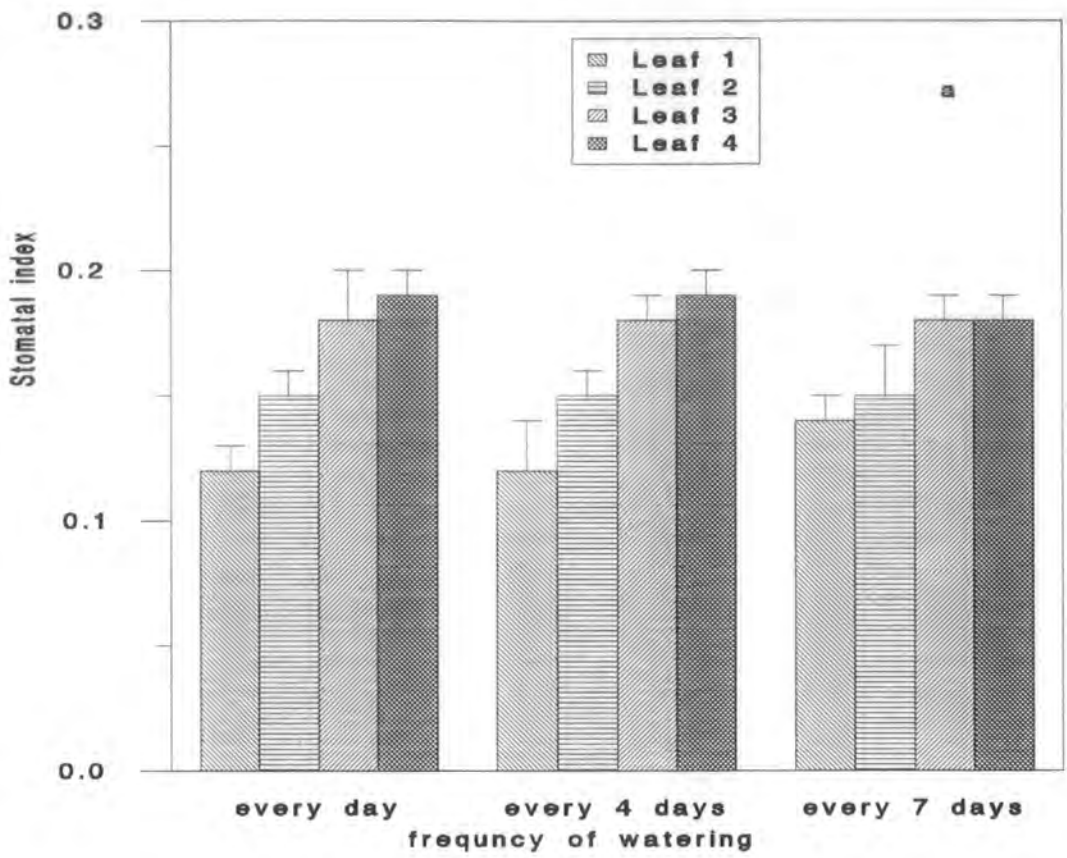
Fig. 3.7 Effects of different watering regimes on the stomatal index of Morgon 845 sorghum seedlings, in the tip region (a), base region (b) and sheath mid-region (c-over).

Fig. 3-7 cont.

125
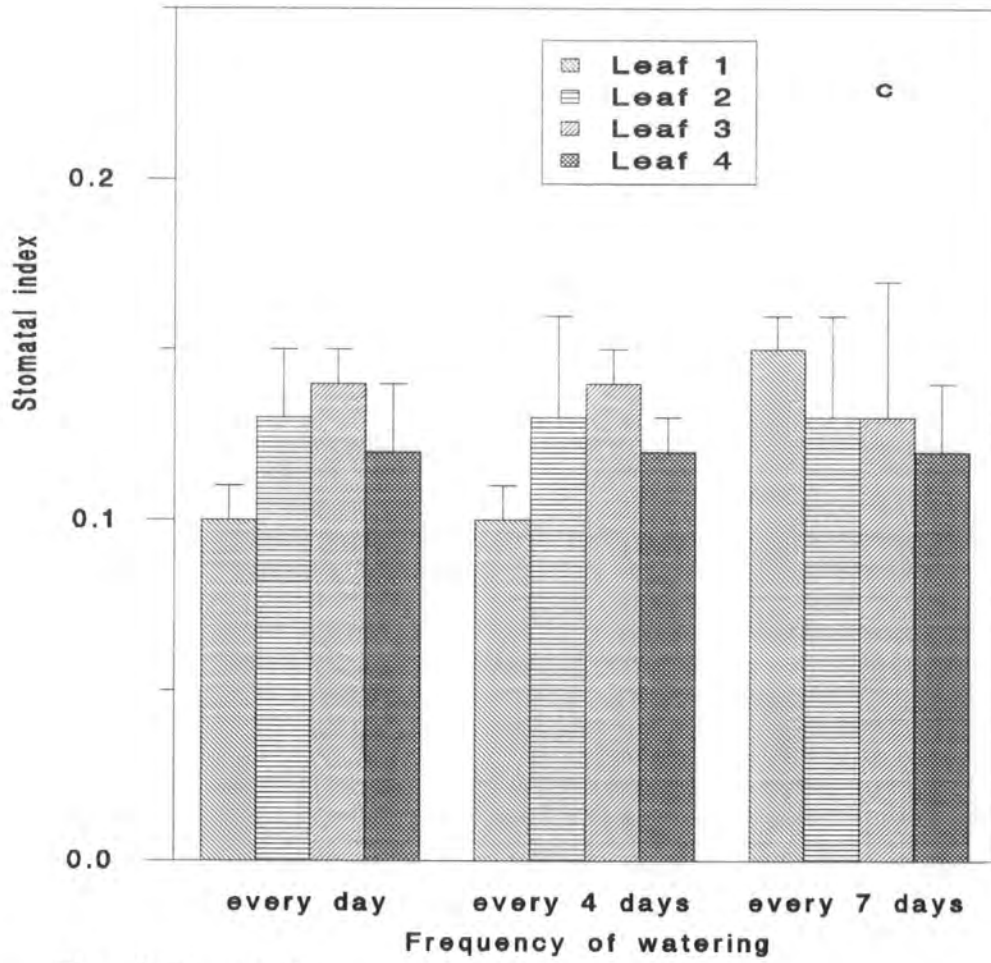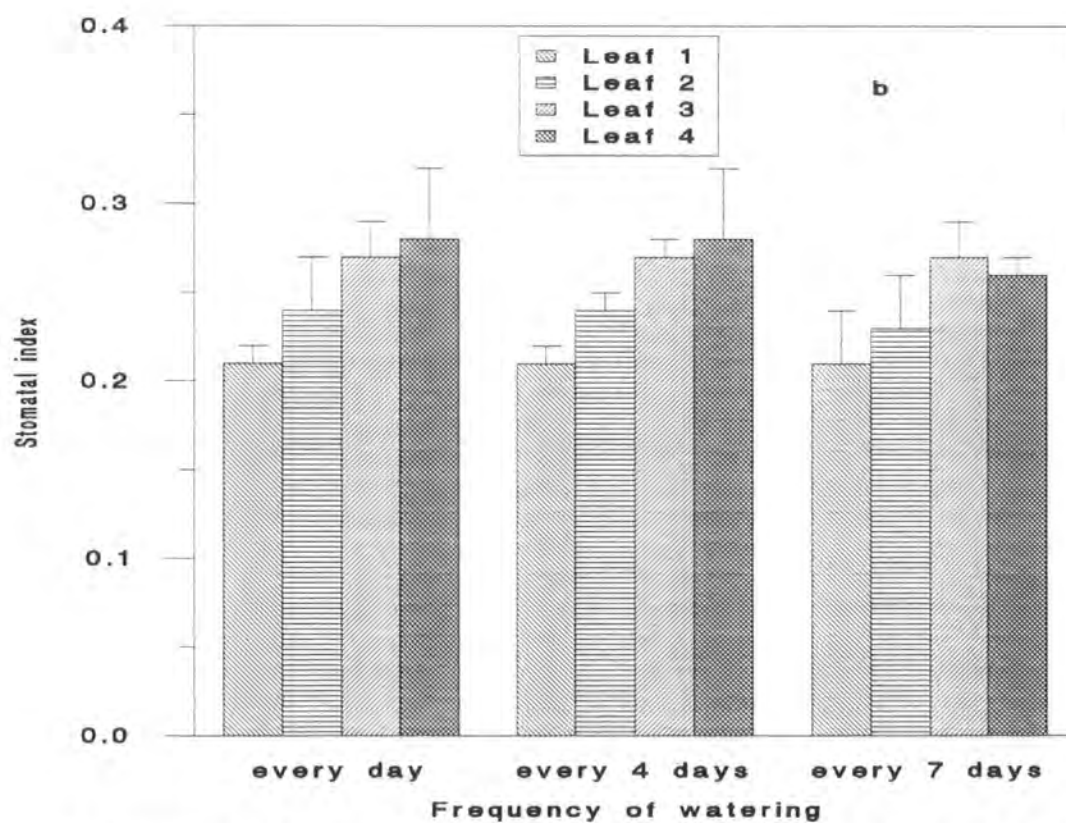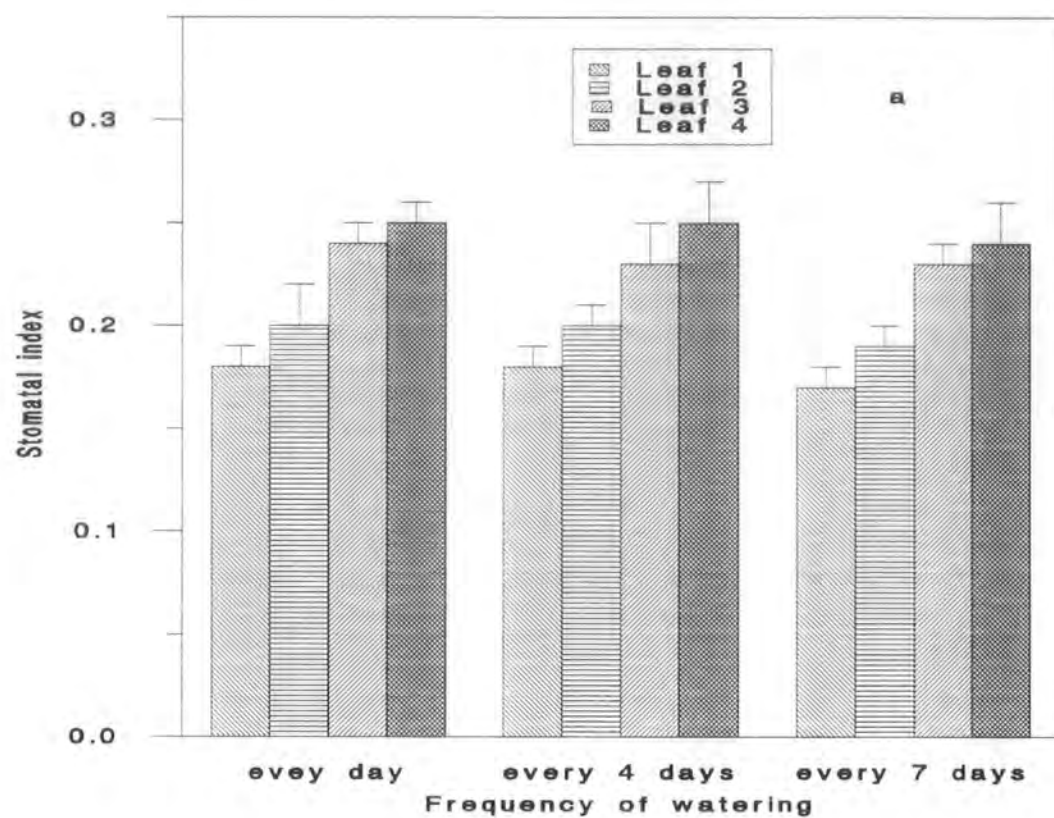
Fig. 3-8 Effects of different watering regimes on the stomatal index of Morgon 856 sorghum seedlings, in the tip region (a), base region (b) and sheath mid-region (c-over).
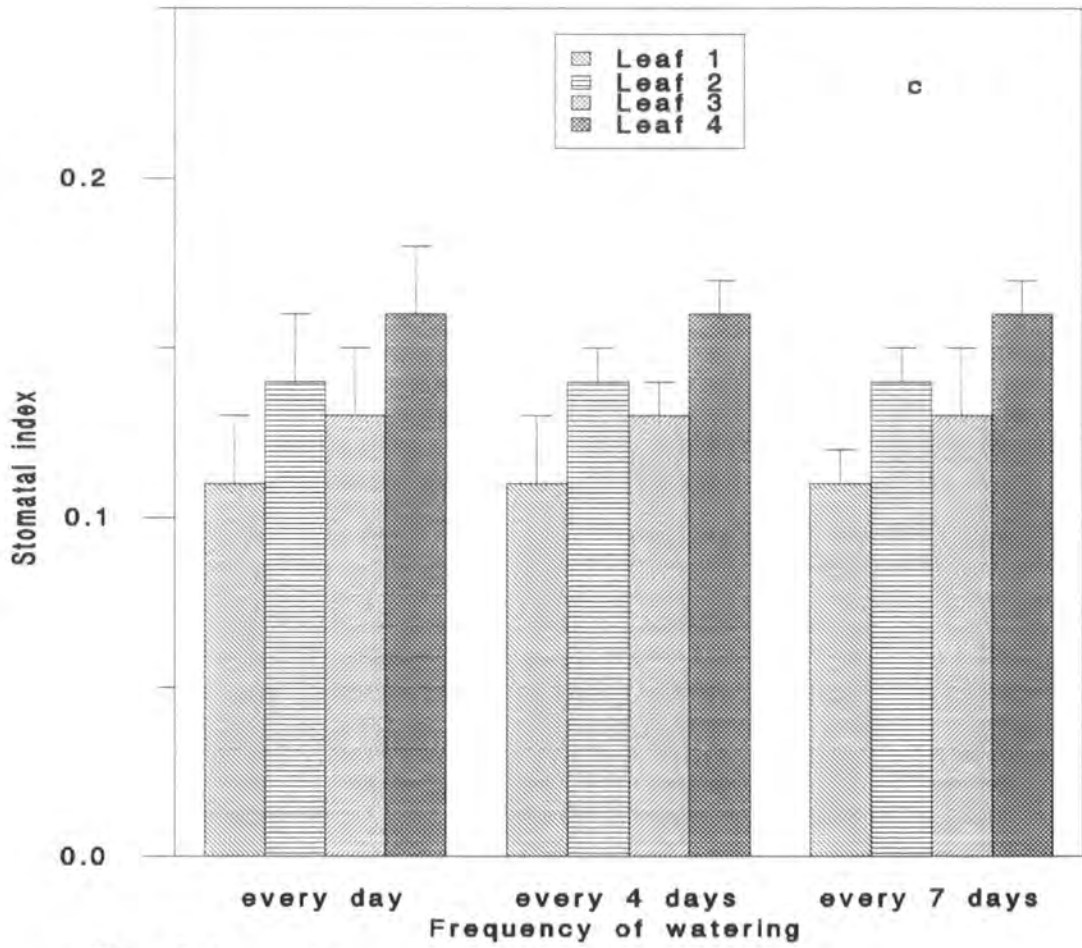
Fig. 3-8 cont.
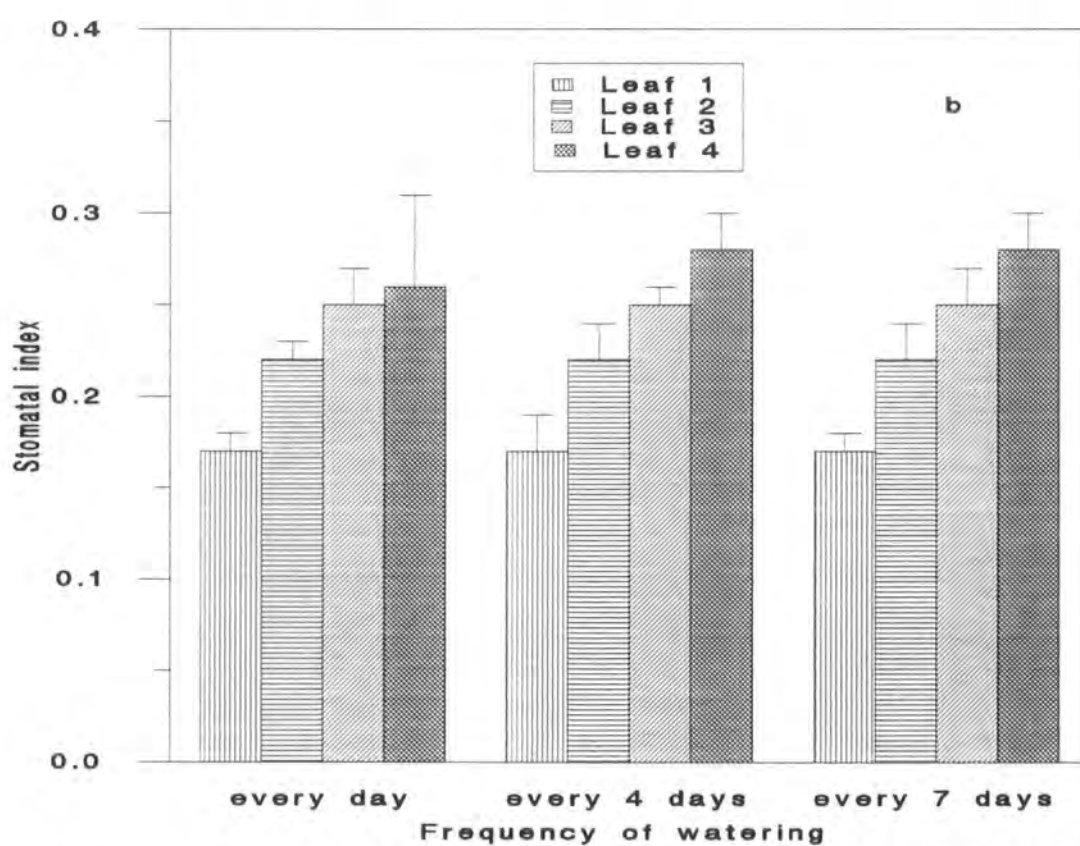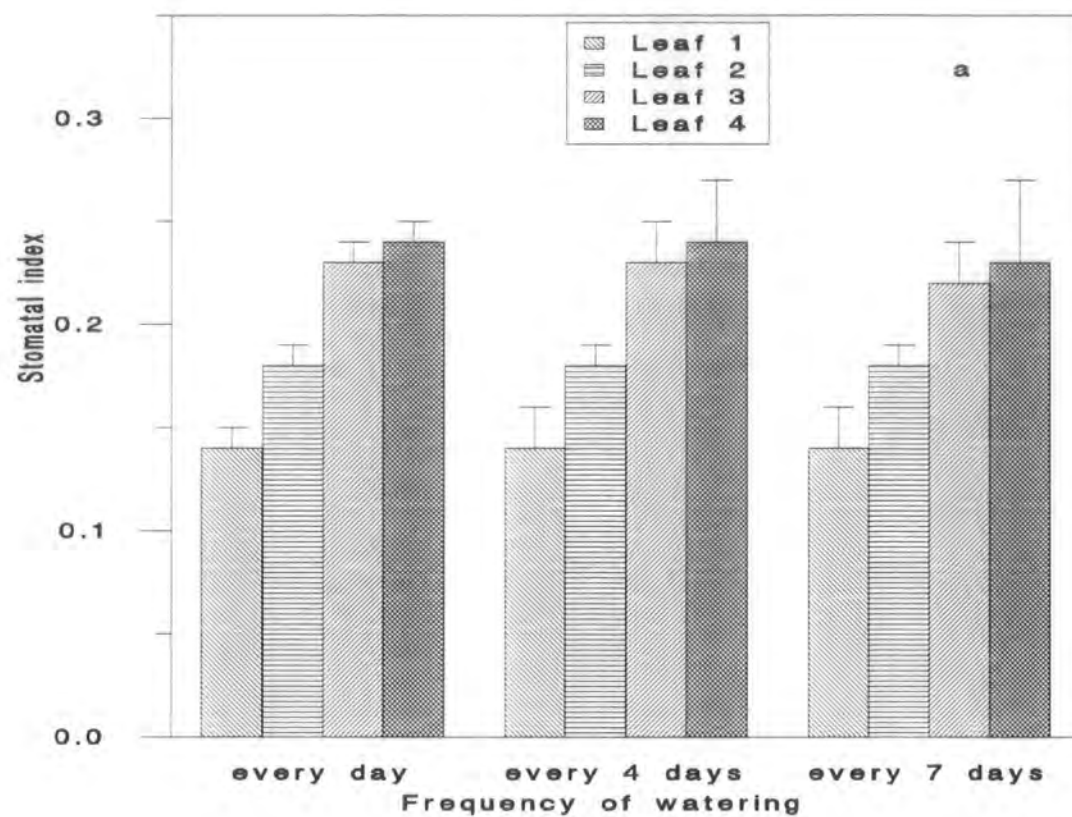
Fig. 3-9 Effects of different watering regimes on the stomatal index of Pioneer 8ss sorghum seedlings, in the tip region (a), base region (b) and sheath mid-region (c-over).
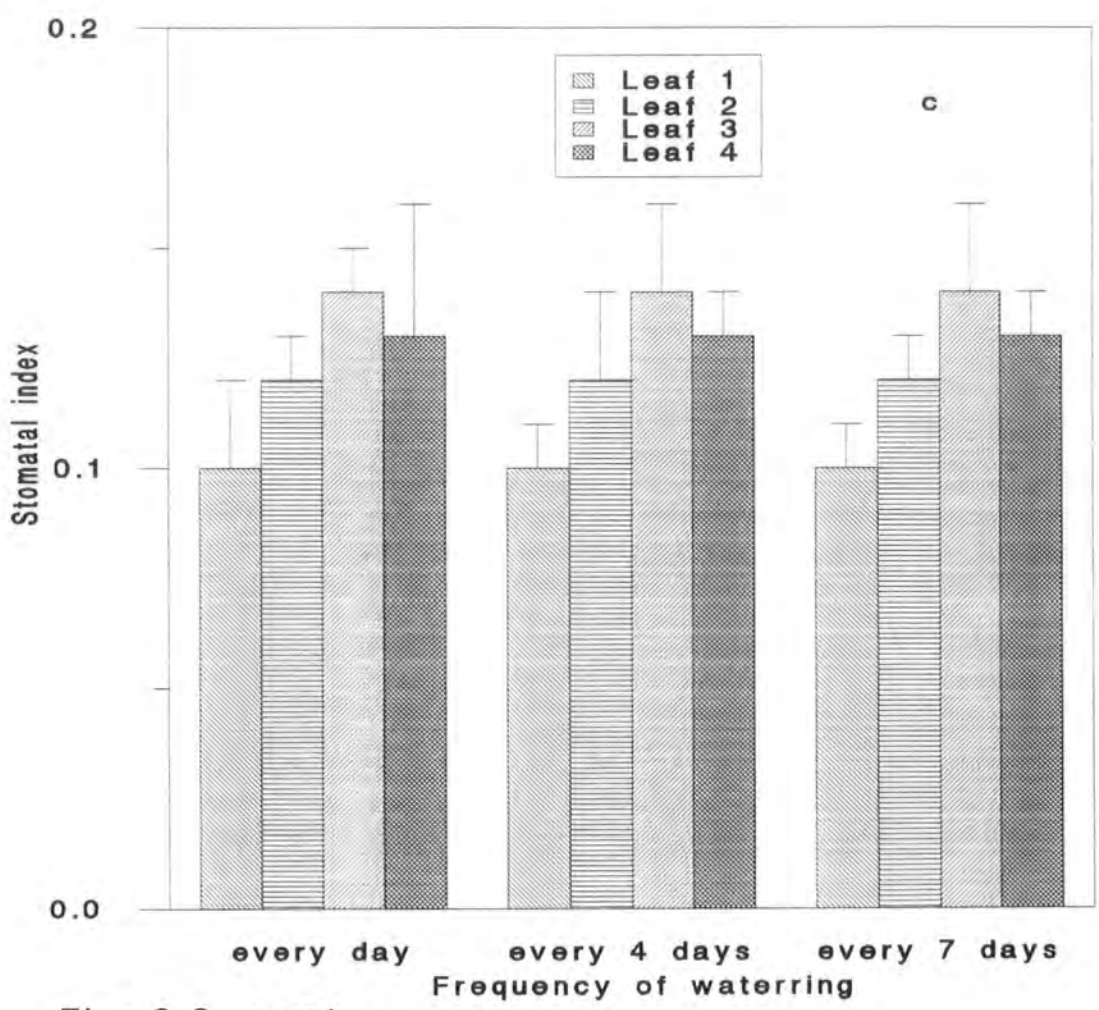
Fig. 3-9   cont.

3.3.2.4 Trichome Index

Calculation of trichome index shows that the 4-day and 7-day watering regimes led to a marked increased in the trichome index in all 3 portions for each leaf, Tables 3.5-3.28. During the treatment watering regimes the extra trichomes initially tended to occur in the rows immediately adjacent to the stomatal rows. Subsequently they occurred more generally over the leaf surface,. More trichomes were produced in response to the 7-day watering regimes than to the 4-day regime which in turn were more than the control.

This trichome response was the same as in wheat but in sorghum there was not a reciprocal decrease in the stomatal index. The increase in trichome index in sorghum was greater than in wheat.

CHAPTER 4

4.1 Plant growth and stomatal characteristics of wheat under water availability manipulated by PEG

The results given in the previous Chapter indicate that water availability has an influence on stomatal development and characteristics in pot-grown wheat plants. In order to investigate this further a more precise manipulation of water regime was used by growing seedlings in hydroponic culture in the presence of osmotic agents, in particular PEG.

Plants were grown as described in Material and Methods for 20 days over which time plant growth was recorded as weight gain. Growth of the leaf lamina was recorded for each successive leaf initiated as increase in length. Stomatal and epidermal cell counts were also recorded over this time period as described previously

4.2 Leaf elongation

Leaf elongation was measured in all treatments at two-day intervals, the elongation zone was located within the sheath of the wheat leaf. Exposure to osmotic solutions brought about an almost immediate reduction of elongation of

the leaf lamina with increased PEG concentrations (limited water availability), leaf elongation decreased proportionally to concentration of osmoticum.

The data in Tables 4.1-4.5 show the effect of various concentrations of PEG on the growth of successive leaves of wheat seedlings. The controls, plants grown in nutrient solution only, showed a steady growth rate for each leaf. Under increasing concentrations of PEG there was a delay in the appearance of successive leaves. This was most apparent at higher concentrations with the result that over the period of the experiment leaf-5 did not emerge under 150 and 200 g/l PEG treatments.

Table 4.1. Effects of PEG concentrations on the length (cm) of leaf 1 of wheat seedlings grown in hydroponic culture (±SE).

DAYS	Control	Concentration of PEG (g/l)					
		50	75	100	125	150	200
2	4.86±0.18	4.90±0.15	4.87±0.13	4.53±0.01	4.40±0.15	4.10±0.06	4.03±0.22
4	5.10±0.06	5.00±0.00	5.03±0.09	4.80±0.10	4.53±0.14	4.20±0.06	4.30±0.15
6	5.63±0.14	5.67±0.09	5.17±0.12	4.93±0.02	4.63±0.17	4.23±0.02	4.53±0.07
8	5.97±0.23	5.70±0.25	5.53±0.07	5.07±0.07	5.17±0.03	5.03±0.03	4.70±0.10
10	6.83±0.07	6.30±0.15	5.87±0.14	5.23±0.02	5.80±0.21	5.20±0.11	5.00±0.01
12	6.90±0.00	6.63±0.12	6.73±0.12	5.43±0.02	6.20±0.06	5.30±0.10	5.13±0.11
14	8.40±0.25	7.97±0.04	7.70±0.20	5.57±0.01	6.20±0.06	5.33±0.02	5.33±0.03
16	9.27±0.37	9.17±0.40	8.57±0.27	6.50±0.06	6.50±0.23	5.43±0.01	5.43±0.09
18	9.80±0.32	9.33±0.34	9.30±0.30	6.90±0.17	6.90±0.00	5.50±0.06	5.73±0.07
20	11.03±0.23	10.00±0.06	9.30±0.30	7.07±0.03	6.90±0.00	5.73±0.13	5.73±0.09
F(df)	139.96(9)	89.62(9)	71.14(9)	81.56(9)	56.99(9)	81.17(9)	29.89(9)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 4.2. Effects of PEG concentration on the length (cm) of leaf 2 of wheat seedlings grown in hydroponic culture (±SE).

DAYS	Control	Concentration of PEG (g/l)					
		50	75	100	125	150	200
2	1.70±0.11	1.03±0.09	–	–	–	–	–
4	3.13±0.09	2.50±0.50	0.97±0.03	0.80±0.06	0.77±0.09	–	–
6	3.90±0.35	3.60±0.15	2.50±0.26	2.53±0.23	2.17±0.22	0.63±0.03	0.80±0.06
8	4.23±0.34	3.87±0.47	3.80±0.42	2.80±0.15	2.40±0.15	1.37±0.01	1.03±0.02
10	5.50±0.26	4.97±0.01	4.10±0.26	3.00±0.11	2.50±0.11	1.47±0.13	1.03±0.17
12	7.43±0.23	6.37±0.47	5.30±0.30	3.43±0.14	2.57±0.12	2.00±0.06	1.83±0.07
14	7.67±0.02	7.13±0.18	6.07±0.01	3.60±0.17	2.90±0.06	2.27±0.02	1.96±0.01
16	8.90±0.58	7.73±0.27	6.77±0.13	4.10±0.06	3.07±0.12	3.57±0.33	3.43±0.22
18	9.67±0.82	9.57±0.31	7.27±0.18	5.10±0.05	5.60±0.06	4.13±0.03	4.73±0.12
20	13.00±0.58	10.76±0.13	10.03±0.11	9.00±0.44	7.50±0.21	6.70±0.06	5.93±0.03
F(df)	82.22(2)	73.03(9)	83.26(9)	62.18(9)	36.24(9)	83.82(9)	370.42(9)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 4.3. Effects of PEG concentration on the length (cm) of leaf 3 of wheat seedlings grown in hydroponic culture (±SE).

DAYS	Control	Concentration of PEG (g/l)					
		50	75	100	125	150	200
2	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–
6	1.43±0.29	0.93±0.01	1.03±0.02	–	–	–	–
8	1.80±0.15	1.47±0.26	1.67±0.12	0.87±0.13	0.77±0.08	–	–
10	2.60±0.21	2.13±0.18	2.07±0.03	1.50±0.21	1.67±0.12	0.70±0.06	0.76±0.08
12	3.30±0.30	3.00±0.06	2.83±0.18	2.87±0.03	2.30±0.17	0.87±0.03	0.80±0.06
14	3.93±0.14	4.03±0.24	3.27±0.32	3.43±0.12	3.67±0.12	1.23±0.14	1.23±0.14
16	5.77±0.59	5.40±0.29	4.43±0.29	4.07±0.03	3.77±0.09	2.03±0.01	2.56±0.18
18	8.87±0.14	7.10±0.61	5.57±0.69	4.60±0.30	4.97±0.03	3.73±0.12	3.30±0.20
20	11.80±0.44	10.53±0.32	9.73±0.12	7.60±0.20	7.23±0.09	5.73±0.13	5.83±0.03
F(df)	70.20(2)	66.73(9)	76.91(9)	83.06(9)	78.71(9)	83.66(9)	75.78(9)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 4.4. Effects of PEG concentration on the length (cm) of leaf 4 of wheat seedlings grown in hydroponic culture (±SE).

DAYS	Control	Concentration of PEG (g/l)					
		50	75	100	125	150	200
2	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–
10	1.23±0.14	0.97±0.03	0.87±0.01	–	–	–	–
12	2.17±0.17	1.83±0.18	1.33±0.17	0.83±0.03	–	–	–
14	3.73±0.22	3.27±0.37	2.13±0.41	0.93±0.02	0.87±0.07	–	–
16	4.23±0.40	4.33±0.30	3.67±0.44	3.57±0.28	3.13±0.18	–	–
18	5.60±0.65	5.13±0.58	4.60±0.25	4.13±0.50	3.20±0.20	0.67±0.03	0.80±0.06
20	6.60±0.51	5.77±0.24	5.40±0.26	5.00±0.32	3.57±0.28	1.63±0.22	1.13±0.12
F(df)	62.59(9)	92.99(9)	81.58(9)	85.61(9)	73.28(9)	57.41(9)	95.02(9)
P≤	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 4.5. Effects of PEG concentrations on the length (cm) of leaf 5 of wheat seedlings grown in hydroponic culture (±SE).

DAYS	Control	Concentration of PEG (g/l)					
		50	75	100	125	150	200
2	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–
12	1.00±0.06	0.93±0.01	0.97±0.03	0.97±0.03	–	–	–
14	2.20±0.15	1.97±0.01	1.53±0.29	0.87±0.04	–	–	–
16	2.80±0.15	2.37±0.23	2.23±0.03	1.33±0.33	0.77±0.09	–	–
18	3.17±0.40	2.80±0.20	3.00±0.00	2.60±0.17	1.33±0.02	–	–
20	4.93±0.03	3.33±0.28	3.63±0.27	3.73±0.38	2.87±0.03	–	–
F(df)	64.83(2)	60.96(9)	58.40(9)	45.24(9)	49.13(9)		
P≤	0.001	0.001	0.001	0.001	0.001		

Consistent with this delay was a suppression of leaf lamina growth. Because leaves emerged at later times under PEG treatment it was not possible to compare the growth of all of them directly against controls. This is because leaf length will be related to length of growing time. However, leaf 1 was present in all the plants at time of transfer to the PEG treatments. It can be seen here that PEG did reduce the growth of the lamina and that the effect an final length (after 20 days) was greater with increasing PEG concentrations. Therefore, for this leaf PEG does reduce the growth of the lamina.

It was assumed that this relationship held true for other leaves and this is indicated where leaves present over the same time intervals e.g., leaf 3 for 10-20 days. Here the rate of growth over this period was 0.82, 0.84, 0.77, 0.61, 0.56, 0.50 & 0.51 cm/day for concentrations control, 50, 75, 100, 125, 150, 200 g/l PEG respectively.

The delay in leaf emergence became greater as higher osmotic potential solutions were used for the growth of the plants. Percentage of leaf emergence was shown to slow more markedly at high concentrations. However, the rate leaf emergence of the plants in PEG was to a certain extent slower than the controls Fig. 4.1.
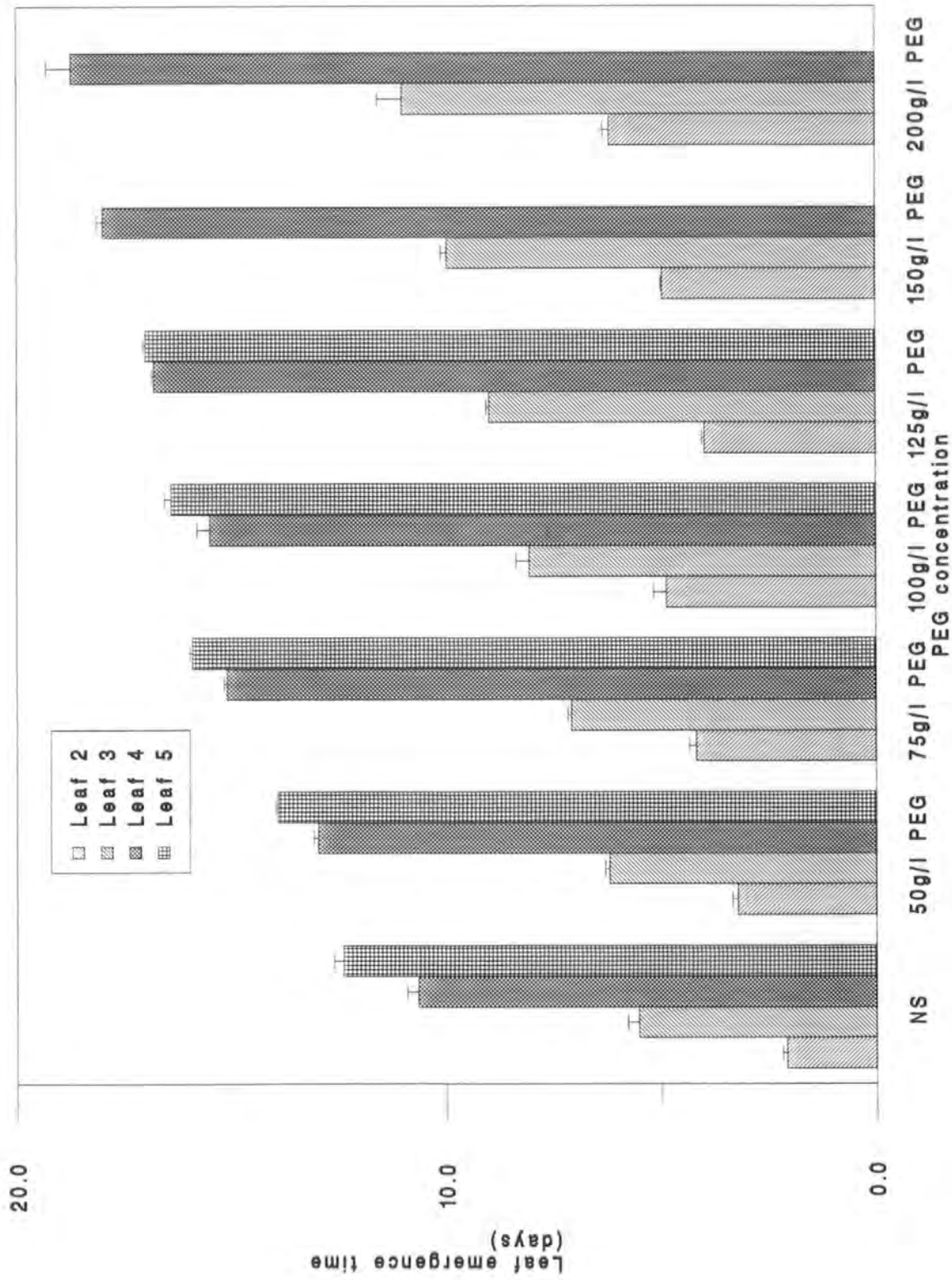
Fig. 4-1 Time (days) taken for leaf emergence of wheat under different concentrations of PEG.

4.3 Root and shoot fresh and dry weight

At the end of the test period fresh and dry weight of both shoots and roots were recorded. All PEG concentrations reduced fresh and dry weight significantly $(P = \leq 0.001)$ in respect of the control. The data indicate, Table 4.6, that the mean of root fresh weight decreased from 0.71 ± 0.10 under control conditions to 0.29 ± 0.03 under 200g/l PEG treatment and the shoot fresh weight decreased from 0.82 ± 0.02 in the controls to 0.44 ± 0.01 under 200g/l PEG treatment. Dry weights of the roots and shoots decreased, in response to water stress. The results in Table 4.6 show that the decrease in root dry weight ranged from 12.5% under 50g/l PEG to 75% under 200g/l PEG and the decreased percentage in shoot dry weight over control ranged from 6.90% under 75g/l PEG to 44.83% under 200g/l PEG. The effects on dry weight indicated that PEG treatment reduces the accumulation of cellular components as well as water for cell expansion.

Table 4.6. Effects of different PEG concentration on fresh and dry weights of wheat seedlings grown in hydroponic culture (±SE).

Treatments	Root fresh weight (g)	Shoots fresh weight (g)	Roots dry weight (g)	Shoots dry weight (g)
Control	0.71±0.10	0.82±0.02	0.16±0.01	0.29±0.01
50g/l PEG	0.68±0.09 (-4.22)	0.80±0.01 (-2.44)	0.14±0.02 (-12.5)	0.29±0.01 (0)
75g/l PEG	0.60±0.03 (-15.49)	0.75±0.01 (-8.54)	0.14±0.01 (-12.5)	0.27±0.01 (-6.90)
100g/l PEG	0.34±0.04 (-52.11)	0.66±0.020 (-19.5)	0.11±0.02 (-31.25)	0.25±0.01 (-13.79)
125g/l PEG	0.32±0.04 (-54.93)	0.62±0.01 (-24.39)	0.09±0.01 (-43.75)	0.23±0.01 (-20)
150g/l PEG	0.31±0.04 (-56.34)	0.55±0.01 (-32.92)	0.06±0.00 (-62.5)	0.21±0.01 (-27.59)
200g/l PEG	0.29±0.03 (-59.15)	0.44±0.01 (-46.34)	0.04±0.01 (-75)	0.16±0.01 (-44.83)
F(df)	28.86(6)	17.27(6)	27.47(6)	15.22(6)
P≤	0.001	0.01	0.001	0.01

The percentage increase(+) or decrease(-) are in parenthesis

4.4 Stomatal density

While all leaves were investigated for their growth responses observations on stomata and epidermal cells were confined to leaf 3 and leaf 5 because they were produced under the PEG treatment conditions. Tables 4.7 & 4.8 show that when plants were grown in different concentrations of PEG there was an increase in stomatal density. This increase was observed in all portions of the leaves examined. However, stomatal density of the basal region was higher than the two other portions. The third and fifth leaves showed comparable stomatal density responses under all the treatments. The increase in stomatal density appeared to be a reflection of decreased cell size, and hence leaf expansion in response to PEG.

Since stomatal density of leaf 3 and leaf 5 were similar in the controls and under PEG treatments this indicates that the plants in hydroponic culture did not show variation in stomatal density in relation to leaf insertion level as was seen clearly in pot-grown plants. A significant ($P = \leq 0.001$) decrease in leaf guard cell length and guard cell width, with increasing PEG level, is shown in Table 4.9. There was a tendency for stomata to be smallest where they were most numerous. The smallest guard cell length $14.38\mu m \pm 2.31$ was found in seedlings under 200g/l PEG, while the largest $22.78\mu m \pm 1.89$ was apparent in the control treatment.

Table 4.7. Effects of different PEG concentration on stomatal density and stomatal index of leaf 3 of wheat seedlings grown in hydroponic culture. Cell characteristics were determined for the tip, middle and base regions of the leaf (±SE).

PEG concentrations	TIP		Middle		Base	
	Stomatal density no/mm²	Stomatal index	Stomatal density no/mm²	Stomatal index	Stomatal density no/mm²	Stomatal index
NS	39.80±2.488	0.15±0.005	37.31±3.480	0.18±0.009	47.26±2.480	0.21±0.005
50g/l	44.77±6.810	0.15±0.007	42.29±3.047	0.18±0.004	52.24±2.702	0.20±0.004
75g/l	52.24±6.410	0.14±0.020	47.26±2.488	0.16±0.007	57.21±2.206	0.17±0.004
100g/l	54.73±2.701	0.12±0.004	49.75±0.000	0.15±0.006	59.70±0.000	0.15±0.007
125g/l	57.21±4.125	0.10±0.003	54.73±3.041	0.13±0.004	62.19±0.001	0.14±0.007
150g/l	62.19±3.486	0.08±0.004	57.21±6.206	0.11±0.003	64.68±5.204	0.11±0.008
200g/l	64.68±4.204	0.08±0.005	64.67±5.699	0.09±0.004	69.65±3.047	0.08±0.003
F (df)	15.13 (6)	27.27 (6)	10.35 (6)	10.35 (6)	19.02 (6)	36.18 (6)
P≤	0.01	0.001	0.05	0.05	0.01	0.001

Table 4.8. Effects of different PEG concentration on stomatal density and stomatal index of leaf 5 of wheat seedlings grown in hydroponic culture. Cell characteristics were determined for the tip, middle and base regions of the leaf. (±SE).

PEG concentrations	TIP		Middle		Base	
	Stomatal density no/mm^2	Stomatal index	Stomatal density no/mm^2	Stomatal index	Stomatal desity no/mm^2	Stomatal index
NS	39.80±5.563	0.12±0.014	37.31±0.001	0.15±0.010	37.31±2.198	0.19±0.003
50g/l	44.77±3.043	0.13±0.005	44.74±4.071	0.14±0.002	42.29±5.817	0.17±0.001
75g/l	47.26±2.488	0.11±0.003	47.26±2.198	0.12±0.003	47.26±2.120	0.15±0.013
100g/l	49.75±0.000	0.09±0.005	52.24±2.260	0.09±0.006	57.21±2.611	0.12±0.003
125g/l	59.70±2.430	0.08±0.002	54.73±2.204	0.08±0.004	62.19±2.619	0.10±0.005
150g/l	NG	NG	NG	NG	NG	NG
200g/l	NG	NG	NG	NG	NG	NG
F (df)	10.98 (4)	29.51 (4)	16.67 (4)	74.54 (4)	24.53(4)	17.23(4)
P≤	0.05	0.001	0.01	0.001	0.001	0.001

Table 4.9. Effects of PEG concentration on the stomatal complex measurements of leaf 3 and leaf 5 of wheat seedlings grown in hydroponic culture (±SE).

PEG	Leaf 3			Leaf 5		
Concentratios	Guard cell length(μm)	Guard cell width (μm)	Epider. cell length(μm)	Guard cell width(μm)	Guard cell length(μm)	Epider. cell length(μm)
NS	22.78±1.89	12.61±2.18	262.47±0.74	12.36±2.54	21.37±0.03	255.06±0.40
50g/l	21.69±0.40	12.29±3.46	262.47±0.68	12.33±2.51	20.32±1.68	254.06±0.42
75g/l	19.79±1.72	11.84±2.29	255.76±0.37	11.76±2.75	19.27±1.80	249.56±0.59
100g/l	19.29±1.85	10.86±1.99	251.83±0.83	10.45±2.50	17.81±0.16	144.05±0.51
125g/l	16.83±2.39	9.60±1.71	248.46±0.58	5.81±1.64	12.89±2.41	135.70±0.36
150g/l	14.39±0.11	8.90±3.17	146.25±0.63	NG	NG	NG
200g/l	14.38±2.31	6.84±2.02	139.04±0.40	NG	NG	NG
F (df)	20.29 (6)	37.02(6)	95.06 (6)	53.43(4)	45.06(4)	58.90(4)
P≤	0.001	0.05	0.001	0.01	0.001	0.001

A similar relationship was noted between guard cell width and growth conditions as was seen for guard cell length. Seedlings grown in 200g/l PEG had a lower guard cell width of 6.84µm ± 2.02 compared with the control values of 12.6µm ± 2.18 in the third leaf. In the fifth leaf a similar effect was seen with 125g/l PEG where guard cell width was 5.81µm ± 1.64 in respect to control values of 21.37µm ± 0.03. In this context stomatal length is of more significant since this is developmentally related, whereas width is a functional response to water availability and other environmental factors which control leaf transpiration.

Following exposure to different concentrations of PEG epidermal cell size decreased in both leaves. Seedlings treated with 200g/l PEG had a lower epidermal cell size of 139.04µm ± 0.40 compared with the controls of 262.47µm ± 0.74 in the third leaf. The fifth leaf followed the same pattern, it had a lower epidermal cell size of 135.70µm ± 0.36 under 125g/l PEG in respect to control values of 255.06µm ± 0.40.

4.5 Stomatal index

It can be seen in Table 4.7 that when plants were grown in a range of concentrations of PEG there was a decrease in the stomatal index. This index was lowest in leaves grown in the highest concentrations of PEG.

The three portions of the third leaf studied showed essentially the same response. However, a higher overall index was noted in the basal region of all treatments. These results show a consistent effect of water limitation, through PEG treatment, on the development of stomatal guard cells in relation to overall epidermal cell development.

When the fifth leaf was examined a similar pattern of response was seen although overall stomatal index tended to be lower in all treatments than for the third leaf, Table 4.8. This difference probably related to variation in stomata in relation to leaf insertion point. Growth of the fifth leaf was prevented at the higher concentrations of PEG and hence no index could be calculated for 150g/l and 200g/l.

4.6 Stomatal density and index in relation to stomatal length

Analysis of stomatal length was made in relation to both the index and the density of stomata. The results in Table 4.10 show that density and index do not give a similar relationship. With increasing PEG concentrations an increase in the ratio of stomatal density/stomatal length was found. This is the result of the increase in density whilst at the same time the stomatal length decreases.

However, the ratio of stomatal index /stomatal length remains more or less constant and is a reflection of a developmental pattern where the decrease in absolute stomatal number is made in proportion to the size of the stomata.

Table 4.10. Stomatal density (SD) (no/mm²) and stomatal index (SI) in relation to stomatal length (SL) of leaf 3 and leaf 5 of wheat seedlings in the base region.

PEG concentrations	SD/SL leaf-3	SD/SL leaf-5	SI/SL leaf-3	SI/SL leaf-5
NS	2.07	1.74	0.009	0.009
50g/l	2.41	2.08	0.009	0.008
75g/l	2.89	2.45	0.008	0.008
100g/l	3.09	3.21	0.008	0.007
125g/l	3.69	4.82	0.008	0.008
150g/l	4.49	NG	0.008	NG
200g/l	4.84	NG	0.006	NG

4.7 Effects of removal of water limitation

Previous results show that treatments with PEG had pronounced effects on stomatal characteristics. The permanence of these effects on the leaves was investigated by returning plants to nutrient solution following PEG treatments. Plants were grown in hydroponics with a range of concentration of PEG for 20 days, after this time the third and fifth leaves were harvested from half of the plants and their stomatal densities and indices were calculated for the basal region. The remaining plants were removed from PEG and transferred to nutrient solution and

were left to grow for a further 10 days. At the end of this time the two leaves under investigation were harvested and the stomatal characteristics were examined as before.

As observed previously, treatment with PEG resulted in an decrease in stomatal index. However, release of water limitation did not result in a recovery of the stomatal index. A similar effect was noted on stomatal density which remained more or less the same as under PEG treatment. This indicates a lack of plasticity once the stomatal patterns has been established. Since no change was detected it can be assumed that cell wall patterns were fixed during PEG treatment.

4.8 Effects of Sorbitol, Mannitol and PEG on the plant growth and stomatal characteristics of wheat seedlings

The objective of this experiment was to investigate the individual influence of PEG, Sorbitol and Mannitol to see if the effect of PEG was as a chemical or as an osmotic agent. To do this wheat seedlings were grown under conditions where 125g/l PEG, sorbitol or mannitol were added to the growth medium until the leaf 3 was fully emerged. In order to assess the effects the final length of leaf 5 was taken as a measure of the treatments on the growth of plants and the stomatal characteristics were recorded for the blade basal region of leaf 3 in order to look

for cellular developmental effects. All three osmotic agents reduced the final leaf 5
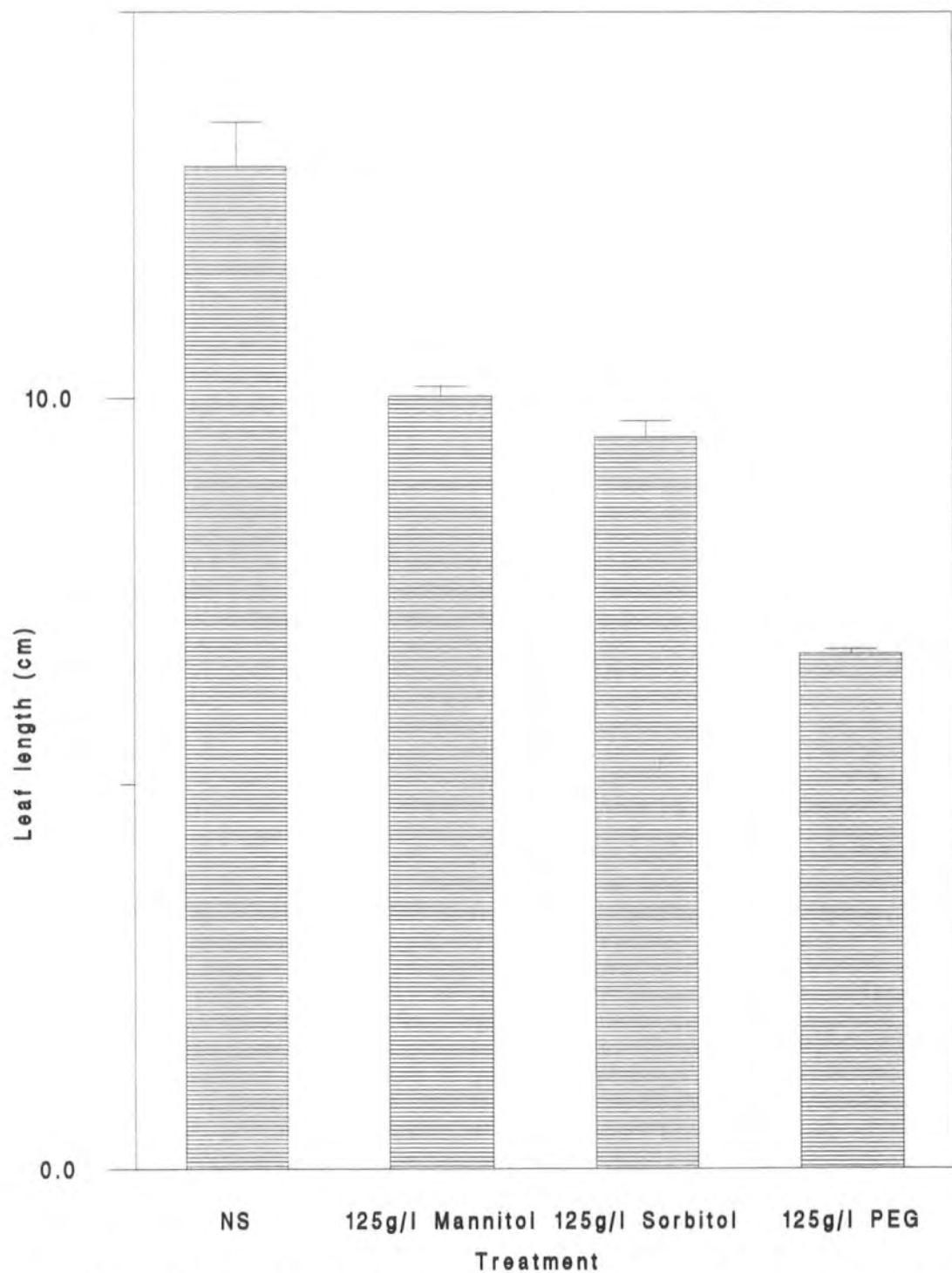
length, Fig 4.2.

Fig. 4-2 Effects of growing wheat seedlings with roots in Mannitol, Sorbitol and PEG on the final leaf blade length of leaf 3.

Table 4.11 shows the effect of 125g/l of mannitol, sorbitol and PEG on stomatal density and stomatal index of the blade basal region leaf 3 in wheat. All the three treatments resulted in increased stomatal density. Which is consistent with previous recorded effects on cell expansion and growth.

All three treatments resulted in a significant (P = ≤ 0.01) decrease in stomatal index. However, a greater effect was seen by the PEG. This may be related to difference in the osmotic potential rather than the properties of the chemicals themselves. Clearly the effect of PEG reported earlier appears to be one of osmotic potential.

Table 4.11. Effect of Sorbitol, Mannitol and PEG on stomatal density (SD) (no/mm²) and stomatal index (SI) of the blade basal region of leaf 3 of wheat seedlings.

Treatments	SD	SI
Control	37.31±0.011	0.21±0.017
125g/l PEG	42.29±6.813	0.15±0.010
125g/l Mannitol	54.73±6.812	0.19±0.012
125g/l Sorbitol	62.19±0.024	0.18±0.001
F (df)	92.56 (3)	18.42 (3)
P≤	0.001	0.001

4.9   Effects of Abscisic acid on stomatal development in wheat seedlings

To investigate the potential signalling from the roots to shoots of seedlings subjected to water limitation use was made of ABA which is known to influence stomata function and is known to be produced in response to water limitation. Seedlings were grown in honey jar cultivation in nutrient solution in the presence of ABA which was root fed. Seedlings were grown for 10 days prior to transfer to ABA and then were grown for a further 10 days before analyses of the stomatal patterns in the third leaf were made.

Seedlings of wheat grown under ABA treatments showed marked reductions in their leaf growth when compared with the controls which were grown in nutrient solution alone, Plate 4.1. These results are consistent with an overall limitation of growth by ABA treatment.

Table 4.12 indicates that when wheat seedlings were treated with ABA decreases were seen in stomatal index in both concentrations used compared to the controls 0.13±0.04 for 2.5mg ABA and 0.17±0.02 for control. The potential that ABA acts as the signal from the roots to alter leaf development is a possibility.

In wheat leaf tissue subjected to water limitation a reciprocal change in stomatal index and trichome index was recorded (Chapter 3). Here it was also noted that trichome index increased under ABA treatments. A significant decrease

in stomatal index from 0.15±0.01 at 1.0mg ABA to 0.13±0.04 at 2.5mg ABA and increase in trichome index from 0.09±0.01 at 1.0mg to 0.11±0.01 at 2.5mg ABA were recorded against the controls values. These results indicates that ABA treatments were able to substitute for water limitation by decreasing stomatal index and increasing trichomes index. The results also indicate a degree of plasticity between the development of the stomata guard cells and the trichomes.

Table 4.12. Stomatal index (SI), and trichome index (TI) of the blade basal region of leaf 3 of wheat seedlings grown under different ABA treatments.

ABA concentration	SI	TI
NS	0.17±0.02	0.07±0.01
1.0mg/ABA	0.15±0.01	0.09±0.01
2.5mg/ABA	0.13±0.04	0.11±0.01
F (df)	18 21(2)	6.32 (2)
P≤	0.01	0.05

Whilst the development of stomata is reduced in ABA, as measured by index, the stomatal complexes appear to develope normally having guard cells and substomatal air spaces Plates 4.2 (a&b). The control tissues was photographed at a magnification of x40 whereas the treated tissues was photographed at x10 magnification.

Plate 4.1. The effect of abscisic acid on the growth of wheat plants in honey jar hydroponic culture.
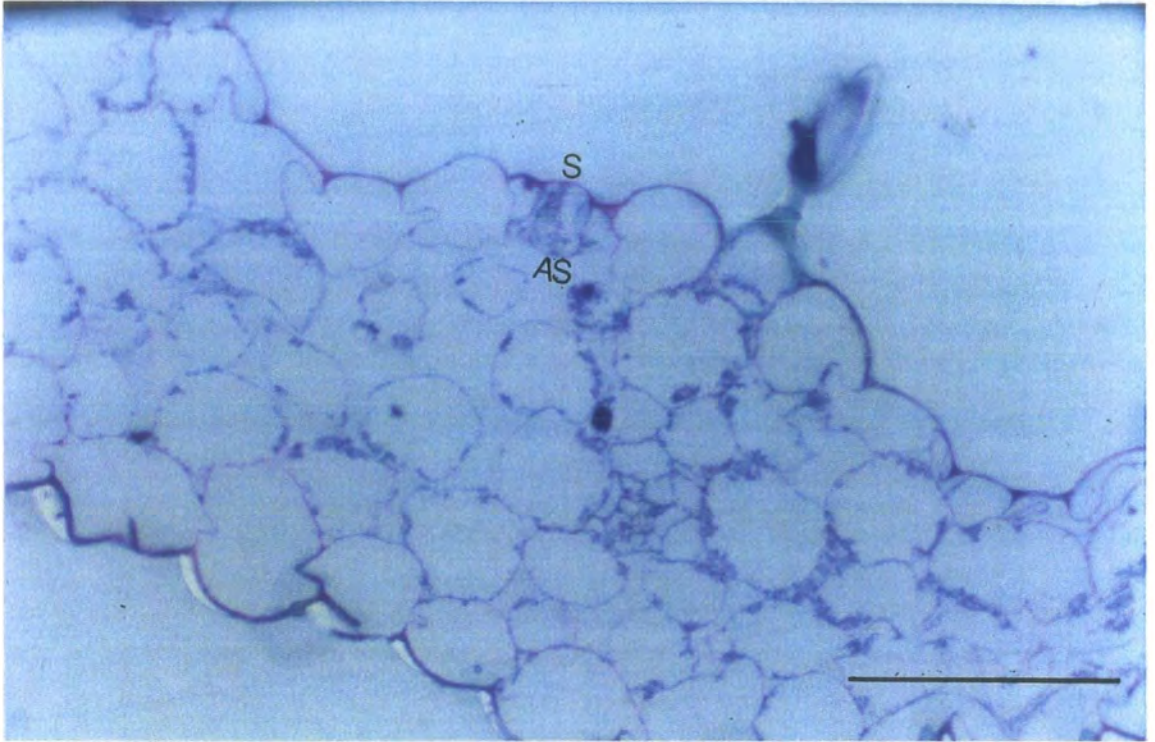

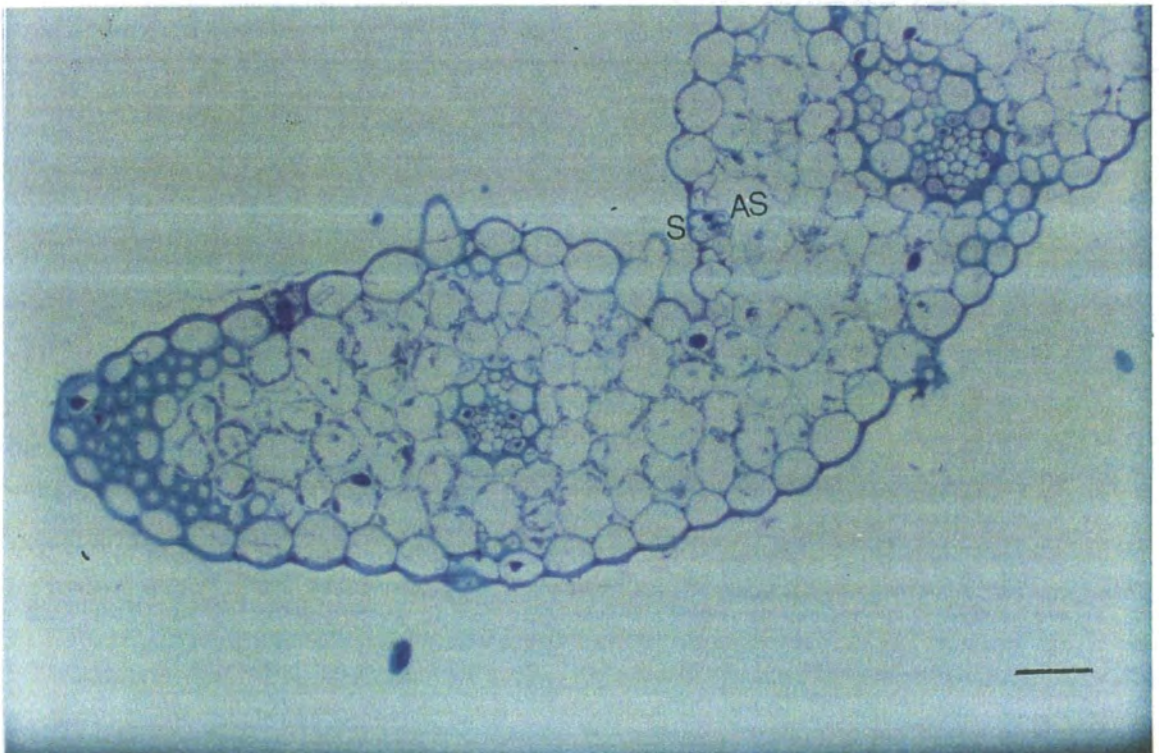1.0mg/l   0.1mg/l   0.01mg/l   control

Plate 4.2. Transverse sections of wheat leaves showing the presence of stomata and sub-stomatal air spaces.

a. Control-plants grown in nutrient solution alone.

b. Plants grown in nutrient solution supplemental with 1.0 mg/l abscisic acid. The black bar = 50μm

4.10   Effects of NaCl on germination and stomata patterns of wheat seedlings

The purpose of this study was to investigate the response of increasing concentrations of NaCl on seed germination and stomatal patterns of the seedlings of wheat, and to determine what level of salinity was detrimental.

Caryopses of wheat were germinated as described in Materials and Methods except that NaCl was added into the growth medium at concentrations ranging from 10 mM to 200 mM. 200 mM was found to be toxic to the extent that the caryopses did not germinate. Therefore the concentration used for the stomatal study was 50 mM which gave a response in terms of growth but did not appear to be totally toxic.

Table 4.13. Effect of NaCl concentration on the caryopses germination of wheat.

NaCl concentrations	Seed germination (%)
0 mM	70.00±17.32
10 mM	70.00±10.00
20 mM	63.33±11.55
50 mM	63.33±15.27
100.mM	26.67±5.77
200 mM	16.67±5.77
F (df)	49.84 (5)
P≤	0.001

Highly significant (P = ≤ 0.001) differences were obtained with respect to germination percentage between control and NaCl stressed seeds. Increasing concentrations progressively delayed germination. The total germination percentage was not affected appreciably by a 10 mM solution of NaCl but was reduced by a 100 mM solution of NaCl. There was a definite delay in germination with increased salinity. At 200 mM concentration of NaCl the total germination of wheat seeds was markedly inhibited when compared to the control Fig 4-3.

Table 4.14 indicate that shoot dry weight accumulation was stimulated under milder saline concentration 0.20±0.001 and decreased under elevated salinity levels in comparison to the control value of 0.10±0.001. The root dry weight decreased against the control at the increasing NaCl concentrations. Fresh weight of roots and shoots data indicated a similar trend with an optimum in 10 mM solution, and lowest at 200 mM.
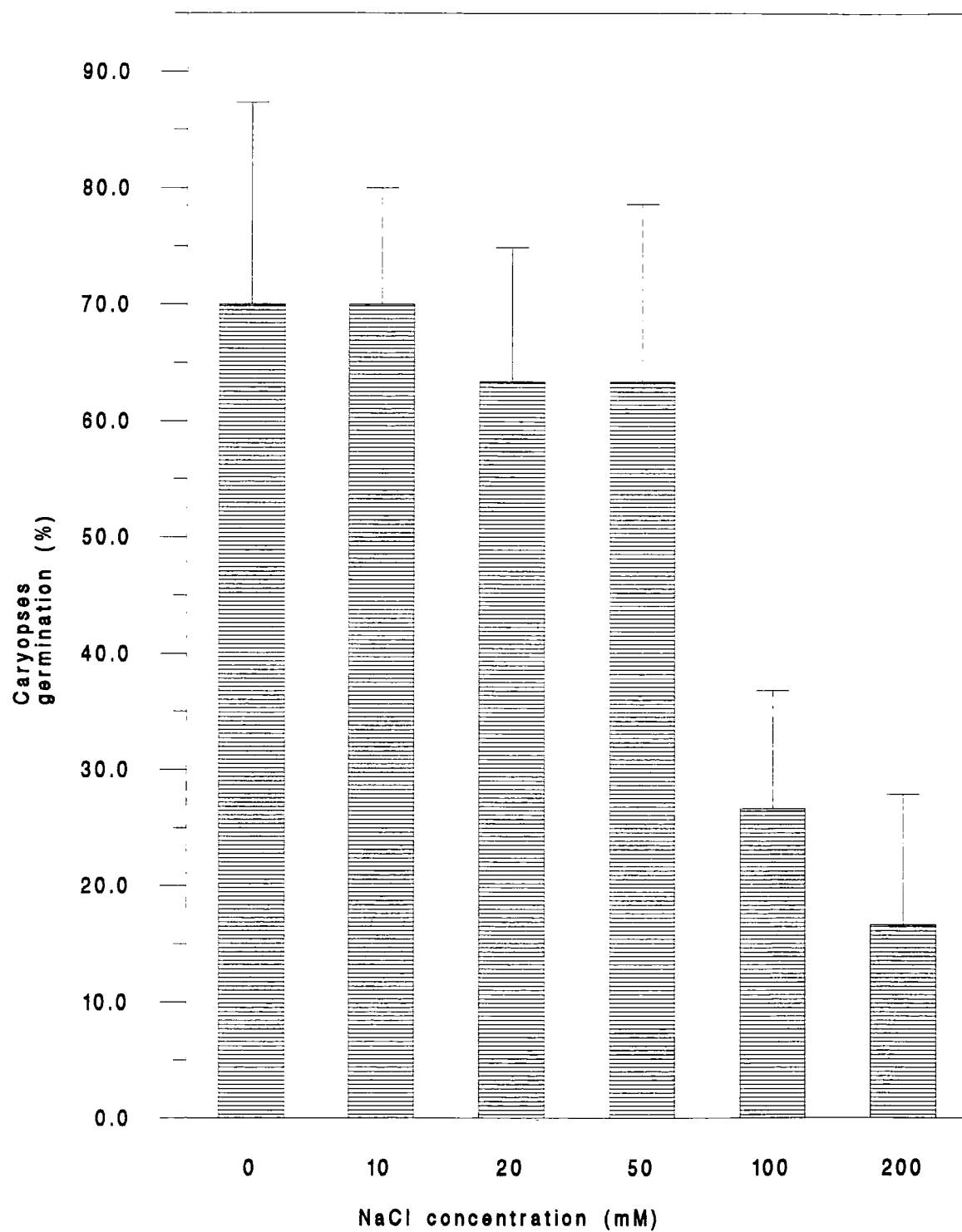
Fig. 4-3 Effect of different levels of NaCl on the germination of wheat caryopses in Petri-dish culture.

159

Table 4.14. Effects of different NaCl concentrations on the dry and fresh weights roots and shoots of wheat grown in hydroponic culture (±SE).

Concentrations of NaCl	Root dry wt. (g)	% inc. (+) or dec. (-)	Shoot dry wt (g)	% inc. (+) or dec. (-)	Root fresh wt. (g)	% inc. (+) or dec. (-)	Shoot fresh wt. (g)	% inc. (+) or dec. (-)
0 mM	0.13±0.000	-	0.14±0.000	-	0.80±0.000	-	1.40±0.000	-
10mM	0.10±0.006	-23.08	0.20±0.001	+42.86	0.94±0.001	+17.5	1.43±0.001	+2.14
20mM	0.09±0.001	-30.77	0.15±0.006	+7.14	0.60±0.004	-25	1.09±0.002	-22.14
50mM	0.08±0.002	-38.46	0.13±0.006	-7.14	0.60±0.003	-25	0.80±0.002	-42.86
100mM	0.04±0.000	-69.23	0.10±0.001	-28.86	0.26±0.040	-67.5	0.41±0.000	-70.71
200mM	nd	-	nd	-	nd	-	nd	-
F (df)	18.02 (5)	-	15.55 (5)	-	29.82 (5)	-	92.62 (5)	-
P ≤	0.001	-	0.001	-	0.001	-	0.001	-

As can be seen in Table 4.15 stomatal density increased slightly in those seedlings which were incubated with their roots in 50 mM NaCl when compared compared with control. This indicates that this concentration of NaCl potentially inhibited cell expansion resulting in a reduction of leaf area and hence increased stomatal density. Stomatal index, however, declined at the same concentration and this indicates and effect on stomatal guard cell development. These effects could be due to either toxicity or water limitation.

Table 4.15. Effect of 50mM NaCl on stomatal density (SD) (no/mm^2) and stomatal index (SI) in the blade basal region of leaf 3 of wheat seedlings.

Treatments	Stoma.density	Stoma. index
Control	32.84±4.123	0.15±0.005
50 mM	37.24±2.112	0.13±0.002
F (df)	13.10 (1)	5.62 (1)
P≤	0.01	0.05

4.11 Osmotic stress effects on wheat using a split root system.

The decrease in stomatal index of wheat leaves when plants were grown in PEG could have been due to a direct effect of osmotic stress on development.

However, it is also possible that the PEG could initiate a signal in the roots to bring about the changes in the leaves. To try and distinguish between these two possibilities a series of split roots experiments were carried out.

Combinations of split roots incubations were performed where each half of the root systems were incubated as follows:-

a- Both halves in nutrient solution

b- Both halves in 125g/l PEG

c- One half in 125g/l PEG and the other half in nutrient solution.

Plants were allowed to grow for 12 days before they were harvested and their root growth, as weight, was recorded along with leaf 3 blade basal region epidermal cell characteristics.

Table 4.16. Split root system of wheat seedlings and roots on fresh and dry weight (±SE).

Treatments	Root fresh weight (g)	Root dry weight (g)
a	0.32±0.03	0.1±0.03
b	0.08±0.02	0.02±0.01
c		
NS	0.28±0.01	0.09±0.01
PEG	0.09±0.01	0.02±0.01

The result in Table 4.16 show that plant roots which were grown up with PEG added to the growth medium grew much less than those in nutrient solution alone. Where one half of the roots was incubated in PEG this also was less than for the nutrient solution alone. Average fresh and dry weight of split roots in nutrient solution was 0.32g±0.03 and 0.1g±0.03 respectively where both halves of the roots were under the same treatment. This compared with a value of 0.28g±0.01 and 0.09g±0.01 for roots in the nutrient solution where the other roots were in PEG. These appears to be no influence of treatment on one half of the root system on the growth in the other half.

In plate 4.3 it can been seen that total root treatment with PEG 125g/l reduced markedly the leaf growth compared with the total root treated with nutrient solution (a). However, when half the roots were in PEG and other in nutrient solution.no effect on shoot growth was seen. This indicates that sufficent water was available to the plant for growth from half the root system.

Plate 4.3. Split root configurations for wheat seedlings
      Left   : left tube-Nutrient solution.
               : right tube-Nutrient solution plus 125g/l PEG
      Middle : both tubes Nutrient solution
      Right   : both tubes Nutrient solution plus 125g/lPEG

Analysis of the stomatal chracteristic were also carried for all of the treatments.

There was a significant increase in the density of stomata in leaf 3 where the whole root system was treated with PEG compared with those treated totally with nutrient solution Table 4.17. However, it can be seen that there was no significant difference in stomatal densities between the control and the plants where half of the root system only was treated with PEG. These plants obviously had significantly lower stomatal densities then the total PEG treatment. The results for total PEG and total nutrient solution treatments are consistent with those reported under 4.4 indicating again an effect of water uptake for cell growth.

Table 4-17. The effect of PEG on the stomatal density (SD) (no/mm²) and stomatal index of the blade basal region of leaf 3 of wheat seedlings grown a split root system.

Treatments	SD	F (df)	P	SI	F (df)	P
NS/NS	33.04±2.201	-	-	0.16±0.001	-	-
NS/PEG	35.24±2.311	5.00 (1)	Ns	0.14±0.001	3.08 (1)	ns
PEG/PEG	46.25±2.342	40.00 (1)	≤ 0.01	0.11±0.012	33.91 (1)	≤ 0.01
		33.14 (1)	≤ 0.01		12.04 (1)	≤ 0.05

NS/NS & NS/PEG Ns
NS/NS & PEG/PEG ≤ 0.01
NS/PEG & PEG/PEG ≤ 0.01

When stomatal indices were calculated it was seen that, as reported under 4.5, total PEG treatment reduced the stomatal index compared with total nutrient solution treatment. However, a slight but not significant reduction in stomatal index was noted for the plants when one half of roots were treated with PEG and the other with nutrient solution. This indicates a slight effect on stomatal development.

Since there was no effect of water limitation on growth this indicates that there was potentially a non-hydraulic influence of the PEG treated root on stomatal development. Such an influence could be mediated through the production of metabolites in the roots such as ABA.

CHAPTER 5

5.1 Effect of PEG on the growth and stomatal characteristics of wheat coleoptiles

In order to attempt to determine which stage in development was the potential point of regulation of stomatal numbers use was made of a wheat coleoptile system which allowed for easier distinction between guard mother cells, epidermal cells and stomata. Trichome development was limited in this tissue and therefore this parameter was not investigated.

Preliminary experiments were carried out on wheat coleoptiles in order to establish the stomatal patterns before detailed manipulative experiments, were performed. The purpose was to investigate the general structure and associated cells of the stomata. To do this the outer epidermis was stripped from coleoptiles of various lengths, mounted in 1% Iodine or 1% Calcafluor, and observed under light or UV fluorescence microscopy respectively. Stomata and GMCs were found localized in narrow strips from 20 mm long coleoptiles, Plate 5.1 and 5.2. GMC's were recongnised as cuboid fluorescence cells which possessed dense cytoplasmic inclusions. The general structure of the stomata was that common to the Gramineae and was the same as for leaves. The guard cells were narrow and elongated and were present in a few rows only away from the tip where they were

more random in distribution. However, the pattern of stomata was not consistent and varied with length, and hence age, of the coleoptiles.

Plate 5.1. Micrograph showing stomata in the middle region of wheat coleoptiles.
Epidermal peel stained with calcafluar and observed under UV microscope. Black bar = 50μm
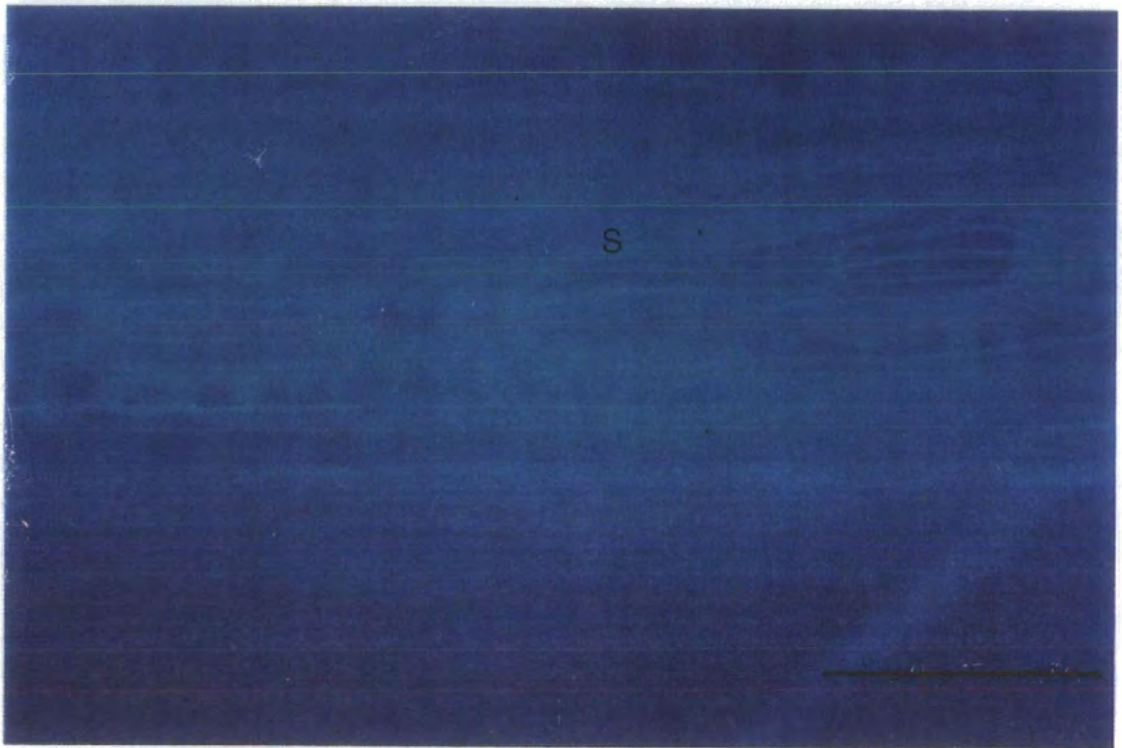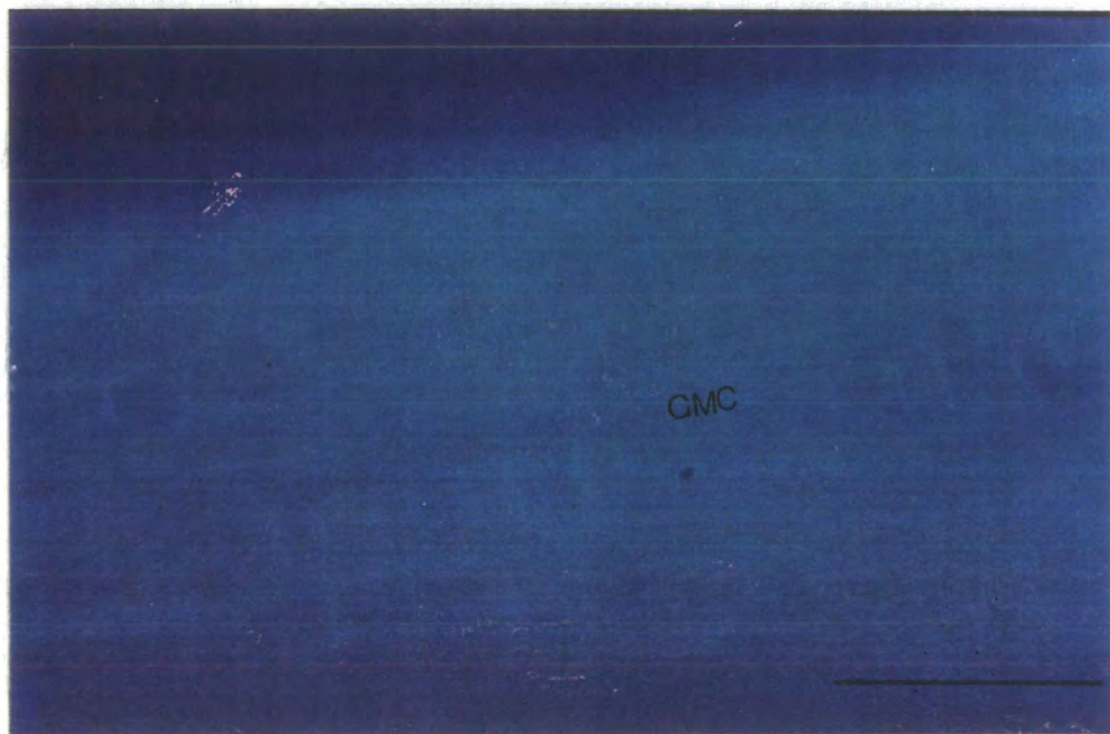S: Stoma.

Plate 5.2. Micrograph showing GMC in the middle region of wheat coleoptiles
Epidermal peel stained with calcafluar and observed under UV microscope
Black bar = 50μm
GMC: Guard mother cell

Coleoptiles were grown to different lengths and then the presence of stomata was recorded for the three equal length segments of tip, middle and base. The fact that the guard cells were more rounded in the tip region and had more pronounced pores may indicate that these were the functional stomata of the coleoptiles. No stomata were observed in the coleoptiles less 10 mm long. However, stomatal density was greater in the tip region than in the middle region Table 5.1. No stomata were detected in the basal region.

Table 5.1. Stomatal density (no/mm^2) and distribution in different lengths of coleoptiles

Coleoptiles size (mm)	Tip	Middle
6 mm	nd	nd
10 mm	15.42±2.69	11.01±1.22
16 mm	19.42±4.13	17.62±2.12
20 mm	23.13±3.10	20.03±2.00
25 mm	25.44±2.01	21.43±1.21
30 mm	34.01±1.12	27.81±4.11

The sizes of guard cells and epidermal cells were less in the tip region than in the middle region Table 5.2.

Table 5.2. Stomatal length and epidermal cell length of wheat coleoptiles.

| Coleoptiles length	Tip		Middle	
	Stomatal length(μm)	Epidermal cell length(μm)	Stomatal length(μm)	Epidermal cell length(μm)
10 mm	26±0.37	156±3.10	35±1.50	169±2.13
16 mm	30±1.51	167±1.14	38±1.51	187±3.19
20 mm	33±2.68	179±3.37	40±3.68	202±2.25
25 mm	37±1.02	198±2.60	42±2.66	216±1.29
30 mm	40±2.86	204±2.97	48±3.67	220±3.33

The stomatal density in coleoptiles increased during the early stages of coleoptile growth in both tip and middle, guard cells size and epidermal cells size also reached a maximum in coleptiles of length 30 mm.

As well as differences in size being detected differences were also noted in the amount and distribution of starch grains revealed by iodine staining, Plate 5.3 (a&b) and Plate 5.4 (a&b). A more dense and even distribution was noted in the more rounded guard cells in the tip region when compared with those in the

Plate 5.3. Micrographs showing stomata in the tip region of dark-grown wheat coleoptiles.
Epidermal peels were stained with iodine
a. Photographed at x20. S: Stoma.
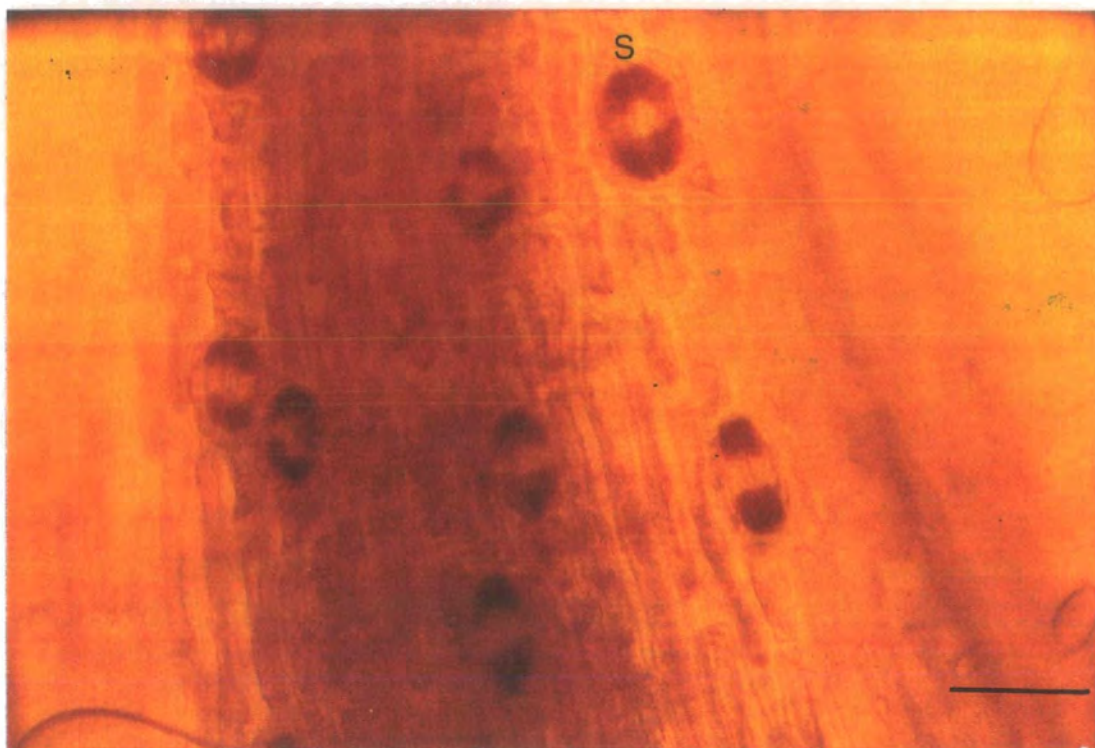
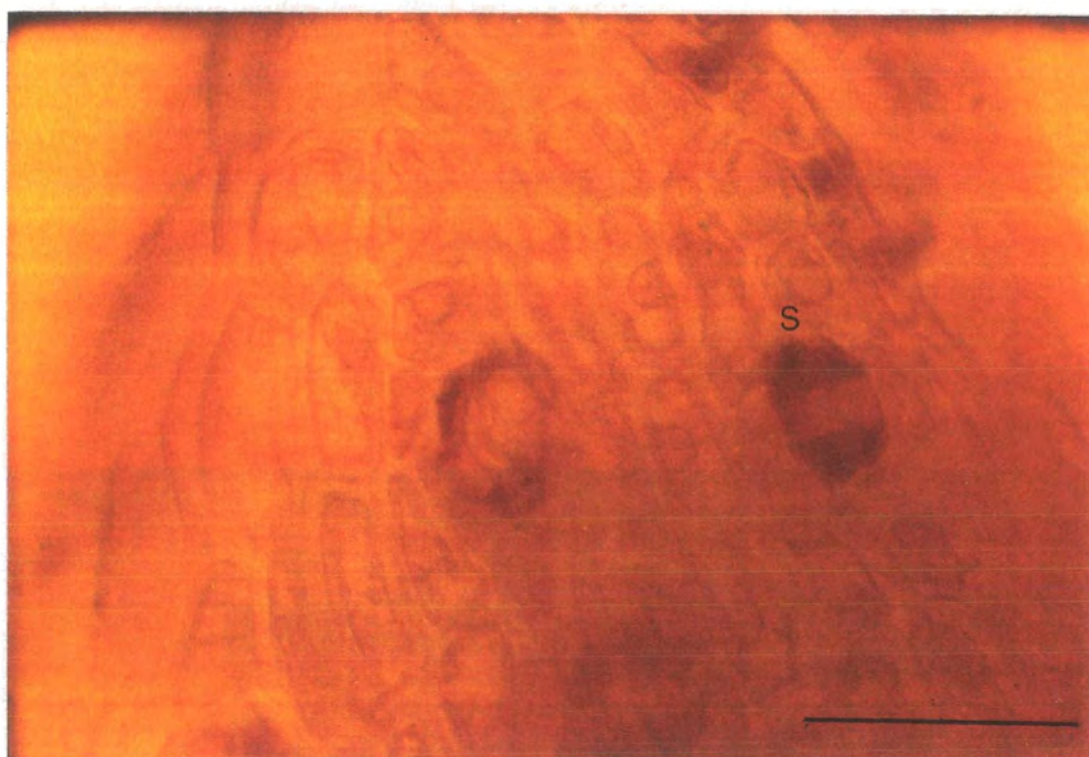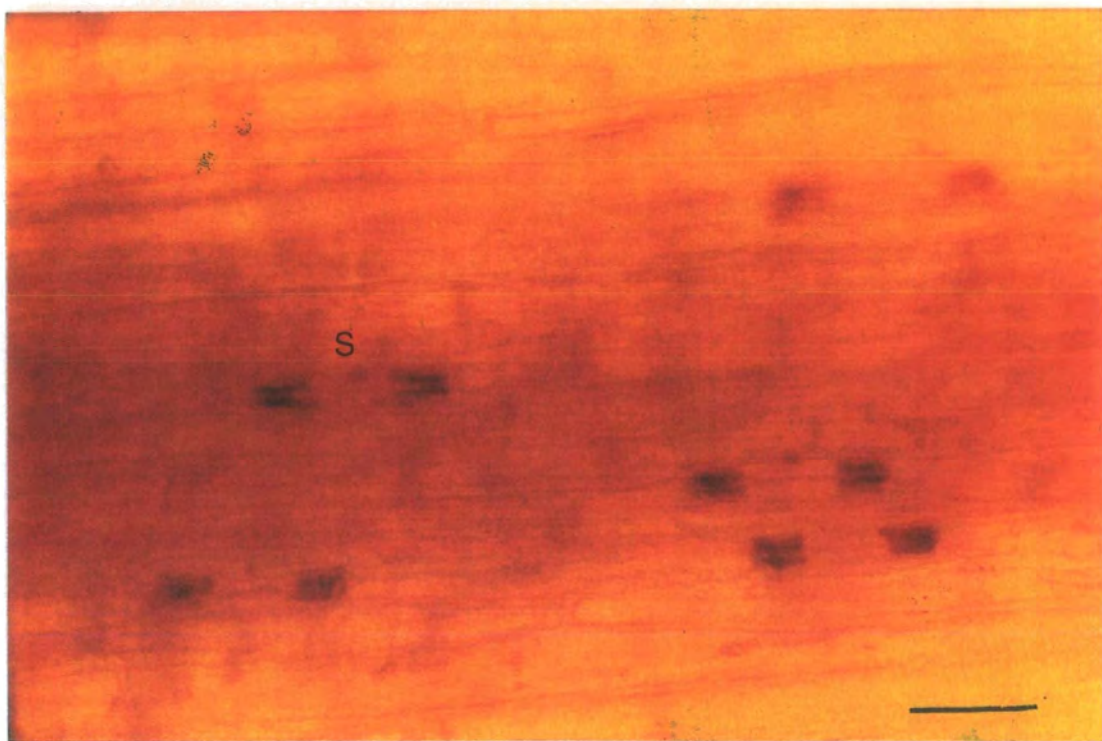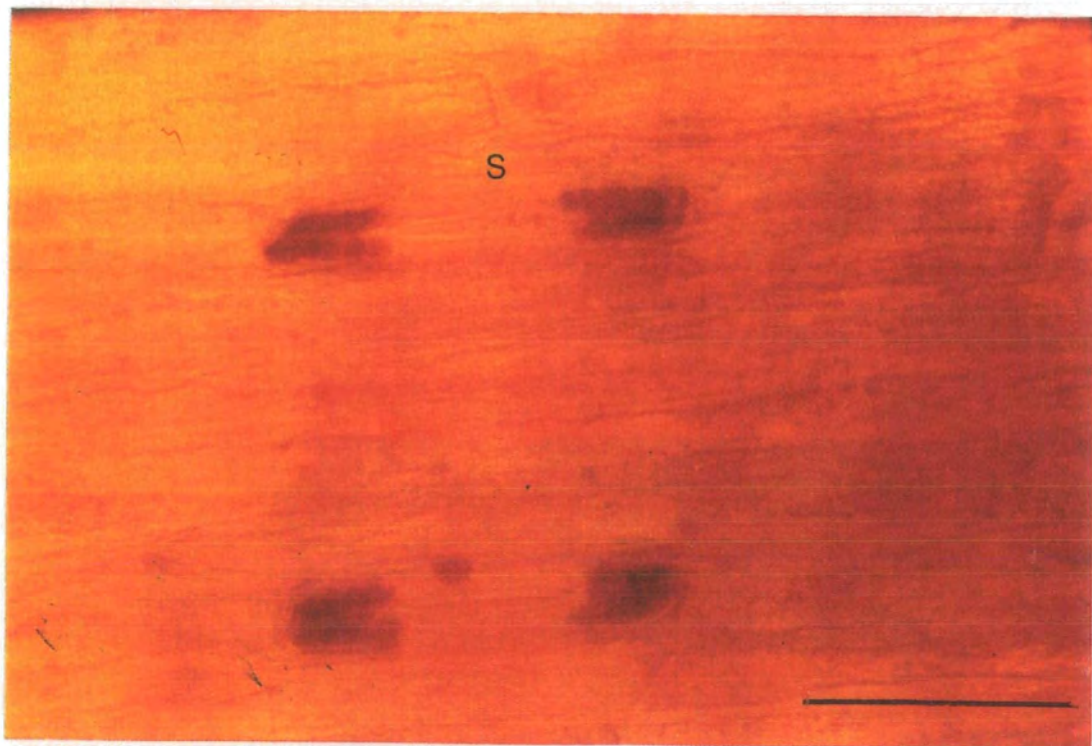b. Photographed at x40. Black bar = 50μm

Plate 5.4. **Micrographs showing stomata in the middle region of dark-grown wheat coleoptiles.**
Epidermal peels were stained with iodine. S: Stoma
a. Photographed at x20. Black bar = 50µm

b. Photographed at x40

middle region where grains were seen to be present associated with the cell ends. This again indicates that those at the tip may have been more functional. Alternatively, it may have been a reflection of the different stages of maturation of the stomata.

Initial experiments were performed to assess the gross effects of PEG treatment on the growth of coleoptiles. As can be seen in Plate 5.5, incubation of newly-germinated seedlings in a range of PEG concentrations resulted in an inhibition of growth of the coleoptiles. Whilst this will involve changes in cell expansion overall cellular patterns could be also be changed as was noted in whole plant incubation experiments (Chapters 3&4).

Plate 5.5. Influence of increasing concentrations of PEG on the germination and early growth of wheat caryopses in the dark.
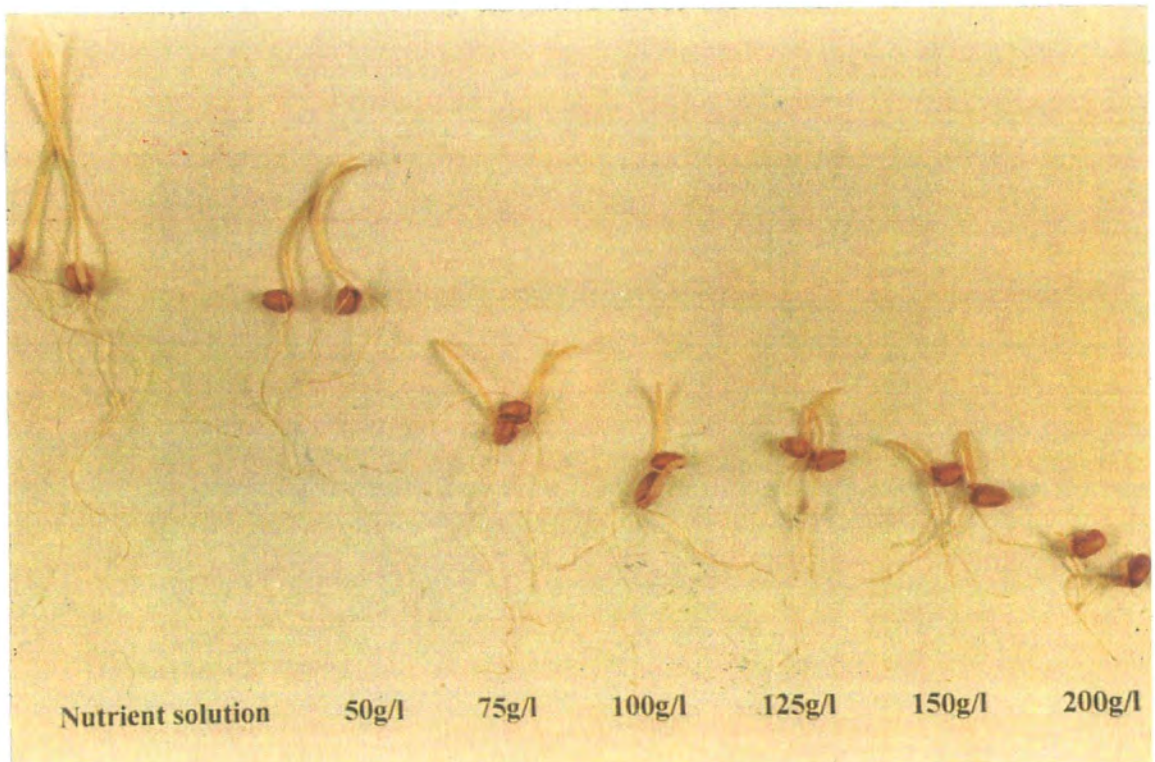
5.2 Effect of PEG on stomatal density, stomatal index, guard mother cell index (GMCI) and guard mother cell density (GMCD) in wheat coleoptiles

As could be predicted, the treatments with PEG resulted in a higher density of epidermal cells which was the consequence of reduced cell expansion and cell size, Table 5.3. As with leaf tissue the increased density of epidermal cells could lead to increased stomatal density, however, this was not as clear in coleoptiles as in leaf tissues.

Table 5.3. Effect of PEG concentration on the epidermal cell density and length in wheat coleoptiles.

PEG concentrations	Epidermal cell density (no/mm^2)	Epidermal cell length (μm)
0	218.06±8.09	229±7.10
50g/l	222.47±8.23	229±9.65
75g/l	233.48±20.43	217±9.17
100g/l	244.20±9.42	208±9.94
125g/l	251.10±12.25	211±10.12
150g/l	305.12±9.12	174±7.94
200g/l	330.40±14.10	153±6.94
F (df)	3.90 (6)	9.75 (3)
P≤	0.01	0.001

Table 5.4 shows that stomatal density in fact decreased slightly. This observation indicates that PEG treatment may have been having an effect on the developmental patterns of stomatal guard cells. There was no reduction in the guard mother cell index or density. These results indicate that, in wheat coleoptiles, guard mother cells production was not influenced markedly by water availability but that subsequent steps of differentiation into guard cells appeared to be a potential point of control.

Table 5.4. Effect of PEG concentration on the stomatal density(SD) (no/mm^2), stomatal index (SI), guard mother cell density (GMCD) (no/mm^2) and guard mother cell index (GMCI) of wheat coleoptiles.

PEG concentration	SD no /mm^2	SI	GMCD no / mm^2	GMCI
0	37.44±0.360	0.14±0.000	39.65±0.417	0.14±0.00
50g/L	33.04±0.857	0.14±0.016	39.12±0.013	0.14±0.011
75g/l	30.84±0.710	0.12±0.015	41.85±0.350	0.15±0.002
100g/l	28.64±0.595	0.11±0.011	48.45±0.583	0.18±0.112
125g/l	26.43±0.013	0.11±0.593	50.66±0.317	0.20±0.123
150g/l	24.23±0.370	0.09±0.326	57.26±0.535	0.20±0.135
200g/l	22.03±0.000	0.07±0.000	62.69±0.000	0.22±0.010
F(df)	4.21 (6)	18.41 (6)	16.84 (6)	4.23 (6)
P≤	0.01	0.001	0.001	0.01

5.3 Effect of PEG on stomatal density and stomatal index in wheat coleoptiles in the presence and absence of roots.

The purpose of this study was to investigate the potential signal from roots to developing coleoptiles which could result in a differentiational change. The wheat grains were incubated for 3 days in nutrient solution in the dark then the seedlings were transferred to Petri-dishes containing nutrient solution with PEG (125g/l) added to them. Three samples of tissues were incubated as follows

(i) Intact seedlings (caryopses, root and coleoptile)

(ii) De-rooted seedlings (caryopses and coleoptile)

(iii) Isolated whole coleoptiles.

Following incubation for a further 4 days the coleoptiles were harvested and examined for cellular patterns. Table 5.5 shows that in all cases the stomatal index was reduced slightly in those coleoptiles which were grown in medium which contained PEG. However, a greater effect on stomatal index was seen when the whole seedlings were incubated. Less decrease in the index was noted when the roots or roots and seed were absent. It would appear that the presence of roots are implicated in the maximum response to water limitation. Thus it appears possible that there was a signal from the roots to the developing coleoptiles associated with the reduction in the development of stomata. In contrast to leaf tissues the density

of stomata, decreased. Stomatal density was slightly lower in intact seedlings (coleoptile, root and caryopses present), than the coleoptiles without roots or roots and caryopses.

Table 5.5. Effect of PEG on stomatal density (SD) (no/mm²) and stomatal index (SI) in wheat coleoptiles.

Treatments	SD (no/mm²)	F (df)	prob.	SI	F (df)	Prob.
Control	33.64±2.017	-	-	0.13±0.002	-	-
125g/l PEG(full plant)	24.20±1.015	10.22 (1)	≤0.01	0.10±0.004	12.86 (1)	≤0.05
125g/l PEG(-r)	28.53±2.016	1.3 (1)	ns	0.12±0.006	1.28 (1)	ns
125g/l PEG(-r&c)	26.13±0.022	8.99 (1)	≤0.05	0.12±0.000	0.54 (1)	ns

-r = coleoptile without root
-r&c = coleoptile without root and caryopses

5.4 Effect of different concentrations of Abscisic acid on stomatal index and guard mother cell index in wheat coleoptiles

In order to assess the potential role of ABA as a signal on GMC index and stomatal index in the coleoptiles, an investigation was performed where intact seedlings were incubated in different ABA concentrations. As was noted in investigations on wheat leaves (Chapter 4) ABA treatments reduced the stomatal index, Table 5.6. However, in coleoptiles ABA also reduced the GMC index as

well as stomatal index. This indicates that ABA treatment potentially altered the differentiation of both GMC and guard cells in the coleoptiles. Reduction of stomatal numbers in this case appears probably to be a result of reduction in GMC.

Table 5.6. Guard mother cell index (GMCI) and stomatal index (SI) of wheat coleoptiles in intact seedlings treated with different ABA concentrations.

ABA concentration	GMCI	SI
0	0.12±0.027	0.12±0.011
0.01/mg	0.12±0.004	0.11±0.005
0.1/mg	0.09±0.017	0.10±0.001
1.0mg/l	0.07±0.000	0.09±0.001
F (df)	18.18 (3)	7.42 (3)
P≤	0.001	0.01

5.5 Effect of light on the growth and stomatal density in wheat coleoptiles.

It has shown that the stomatal density in a number of plants can be influenced by the light level under which they were grown.

In order to study this in wheat an investigation of the effect of light on the growth and stomatal density of the coleoptiles was made. Caryopses of wheat

were sown in Petri-dishes in water. Batches of Petri-dishes were then incubated in combinations of light and dark as described in the Materials and Methods.

Table 5.7 shows that exposure of the newly germinated seedlings to increasing light periods (from 0 to 7 days) resulted in a reduction in the growth of the coleoptiles. An increased in light period produced shorter coleoptiles.

Table 5.7. Effect of light and darkness periods on wheat coleoptiles length.

Darkness & Light periods (days)	Coleoptile length (mm)
0D/7L	22±0.19
1D/6L	25±0.07
2D/5L	25±0.02
3D/4L	27±0.06
4D/3L	27±0.04
5D/2L	28±0.04
6D/1L	28±0.01
7D/0L	30±0.01
F(df)	2.48 (7)
P≤	0.05

At the same time as the coleoptile lengths were being reduced a reduction in stomatal density was also noted in both the tip and the middle regions. This could be due to an arrest of stomatal development and is consistent with the treatments which reduced coleoptile growth.

Table 5.8. Effect of light on stomata density (no/mm^2)in the tip and middle

regions of wheat coleoptiles

Darkness and light periods	Tip	Middle
0D/7L	25.84±2.20	19.62±2.99
1D/6L	25.24±1.21	19.04±2.00
2D/5L	26.44±2.69	22.83±1.20
3D/4L	26.85±2.00	23.03±1.21
4D/3L	28.85±3.19	24.23±2.00
5D/2L	28.25±2.23	26.23±1.23
6D/1L	30.05±2.00	26.03±3.11
7D/0L	32.65±1.11	29.03±2.03
F(df)	2.53 (7)	3.48 (7)
P≤	0.05	0.05

Table 5.8 shows that there was a slightly increase in stomatal density under

a different periods of light and darkness on the tip and middle region of the

coleoptiles.

CHAPTER 6

6.1 Root pigmentation in wheat

During growth of wheat plants in honey jars in the presence of PEG it was noted that a yellow pigmentation developed in the roots. This pigmentation was not observed in plants grown in the nutrient solution alone i.e. without water limitation (Plate 6.1). Here the roots remained white. Reports have been made of the presence of similar pigmentation of roots during soil water stress treatment, Zgurouskaya (1955). However, there was no report of any investigations into the nature or biology of the pigment.

Apart from the initial observations in this study on wheat the chemical nature of this pigmentation under PEG treatment was not clear. The possibility existed that it may have been due to the presence of carotenoids or xanthophylls which are known to be potential biological precursors for abscisic acid (Neill *et al* 1984). As has been shown in previous Chapters Abscisic acid appears to be involved in the water stress response of wheat where it can alter stomatal patterns. The build up of the pigment could therefore have been related to the production of abscisic acid under stress treatment. The nature of this pigment was examined further in terms of its chemistry and physiology.

Plate 6.1 Roots of wheat seedlings showing the presence of pigmentation in response to growth in PEG. Plants on the right were grown with their roots in nutrient solution. The other set were grown in nutrient solution supplemented with 125g/l PEG.
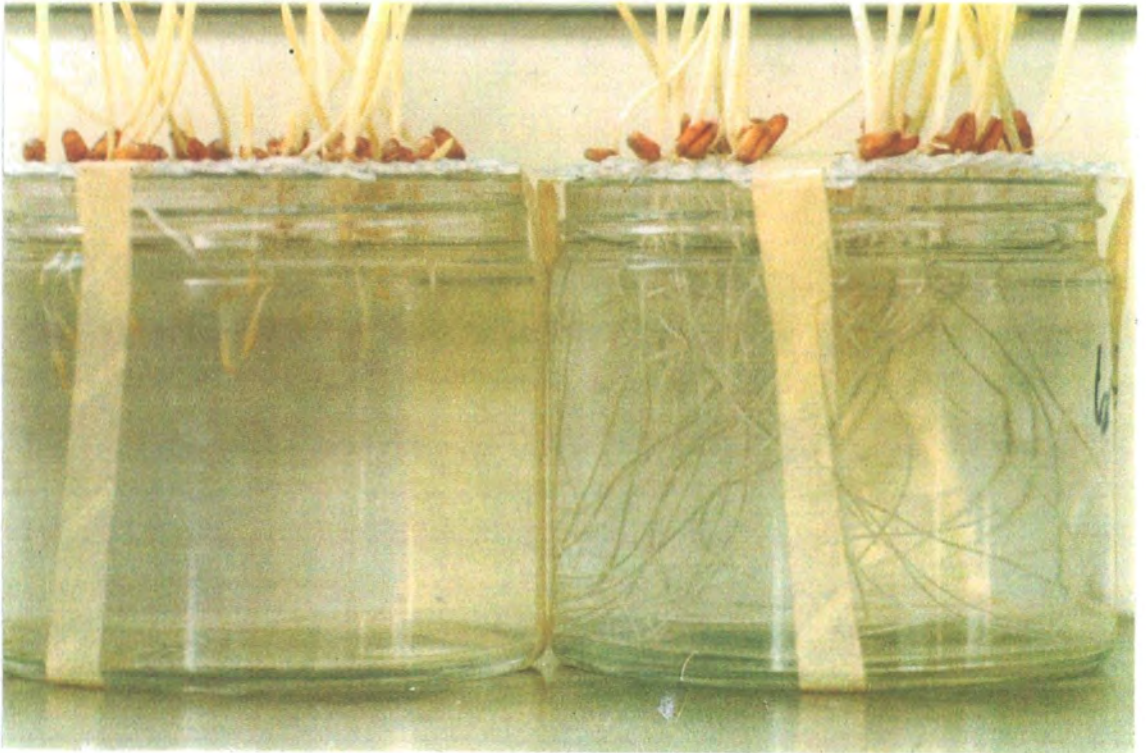
Seedlings were grown in honey jar hydroponic culture with or without PEG added to the nutrient solution, in a light growth room as described in Material and Methods. Two concentrations of PEG were used, 125g/l and 200g/l. Extraction of the pigment from harvested and washed roots was made routinely from plants at time intervals from 3 day to 16 days after the start of experimental treatments.

Initial extractions of the suspected pigment were made using diethyl ether which is known to solubilise carotenoids, details in the Material and Methods. Total extraction of the root pigment was achieved by this technique indicating that its solubility properties in ether were similar to carotenoids.

The root pigment was also soluble in absolute ethanol. Fig 6.1 (a) shows the absorption spectrum, in diethyl ether, of a crude diethyl ether extraction of roots of wheat plants grown in nutrient solution. This can be compared with an extract of roots grown in nutrient solution supplemented with 125g/l, Fig 6.1 (b). Absorption peaks were noted at 423nm and 450 nm for PEG treated material. No specific peaks were noted at these wavelengths for the control tissue extractions. These absorption patterns for pigmentation appear therefore to be a specific response to PEG treatment. The pigment accumulated within 3 days of transfer of the plants directly to PEG. Only a slight increase was seen in the amount of extracted pigment when the plants were grown for up to 16 days in the same

Fig.   6.1. Absorption spectra, in diethyl ether, of crude a diethyl ether extraction of wheat roots.

a. Plants grown for 10 days in nutrient solution
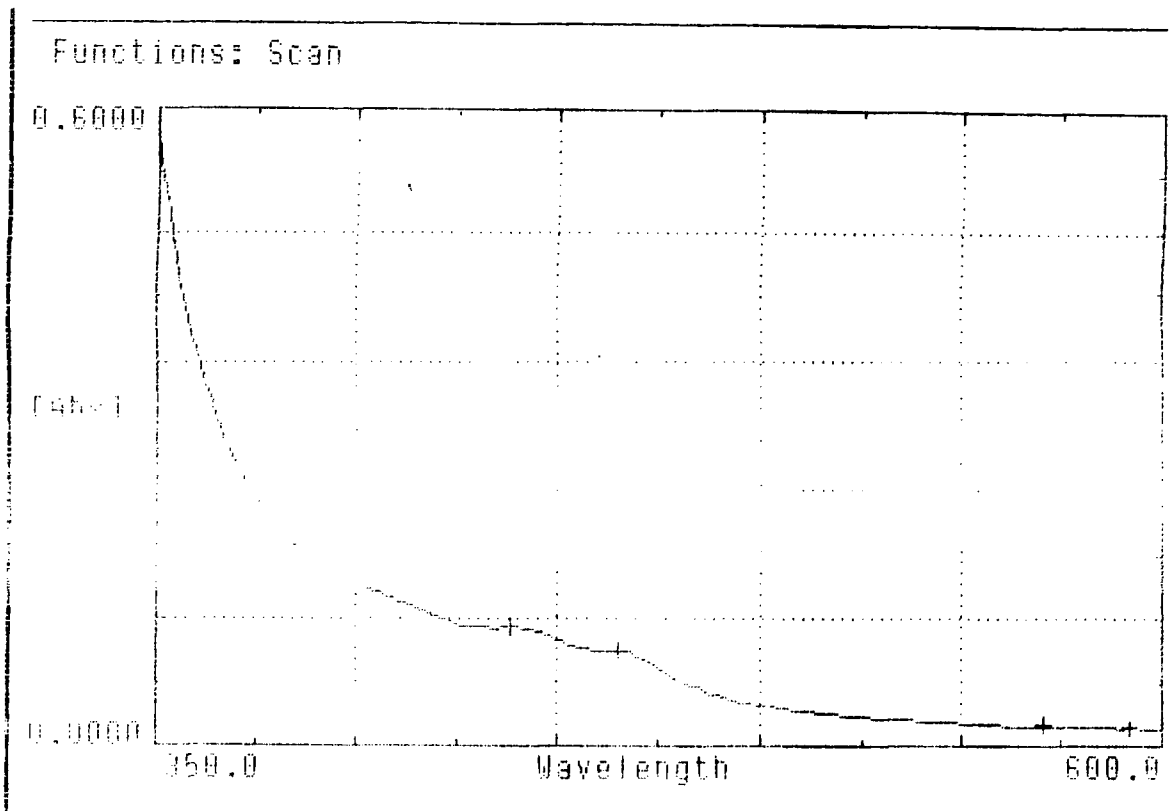
b. Plants grown for 10 days in nutrient solution supplement with 125g/l PEG.
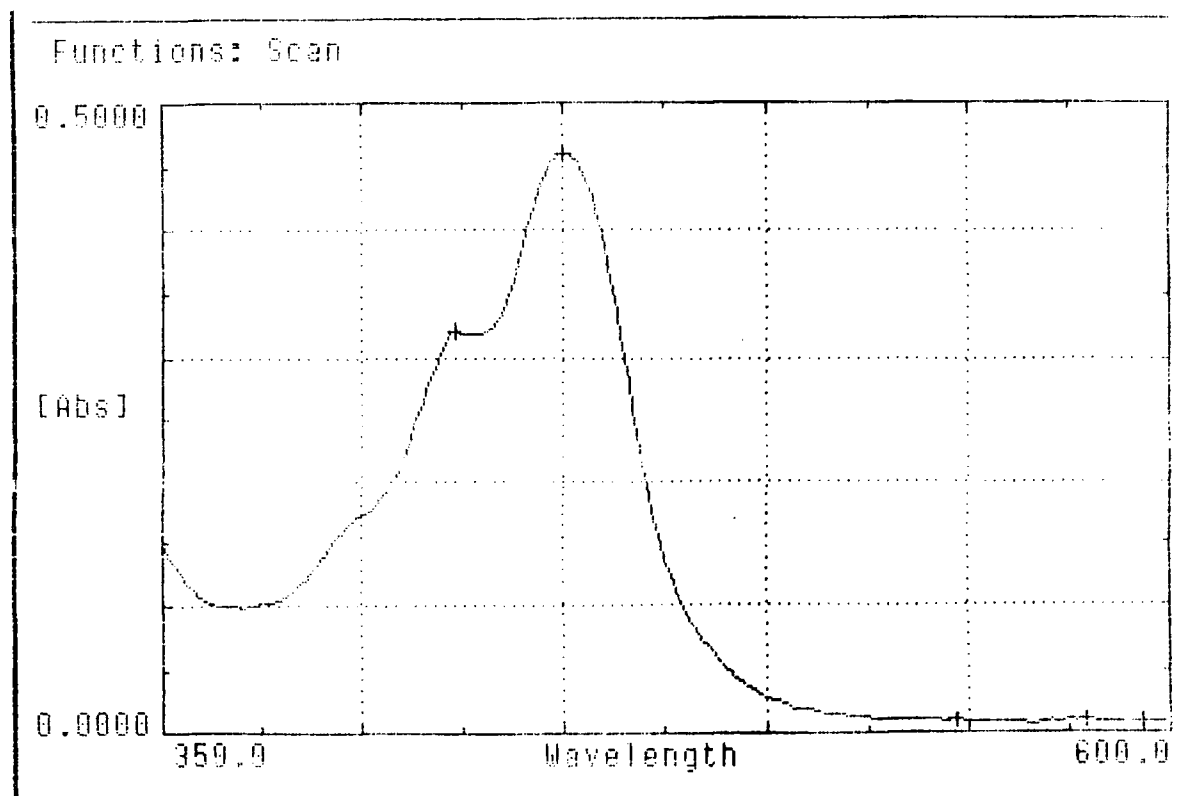
treatment, Fig 6.2 (a&b). Pigment also accumulated in roots of plants which were transfered to PEG after first having been grown in nutrient solution. However, here there was a delay in the appearance of the pigment with it becoming apparent after 5 days. Plants grown in nutrient solution first had mature roots which did not respond in terms of pigment accumulation at the same rate as roots initiated in PEG.

When plants were grown in higher concentrations of PEG no increase in the amount of pigment, based on maximum peak height per mg fresh weight, was noted. Fig 6.3(a&b) show the extracted root pigment spectra for plants grown in 125g/l and 200g/l PEG respectively, in this case ethanol was used as the solvent. The absorbtion spectra are essentially the same for the pigment dissolved in either ether or absolute ethanol.

Fig.   6.2. Absorption spectra, in diethyl ether, of an crude diethyl ether extract of wheat roots.

a. Plants grown with their roots in 125g/l PEG for 3 days.
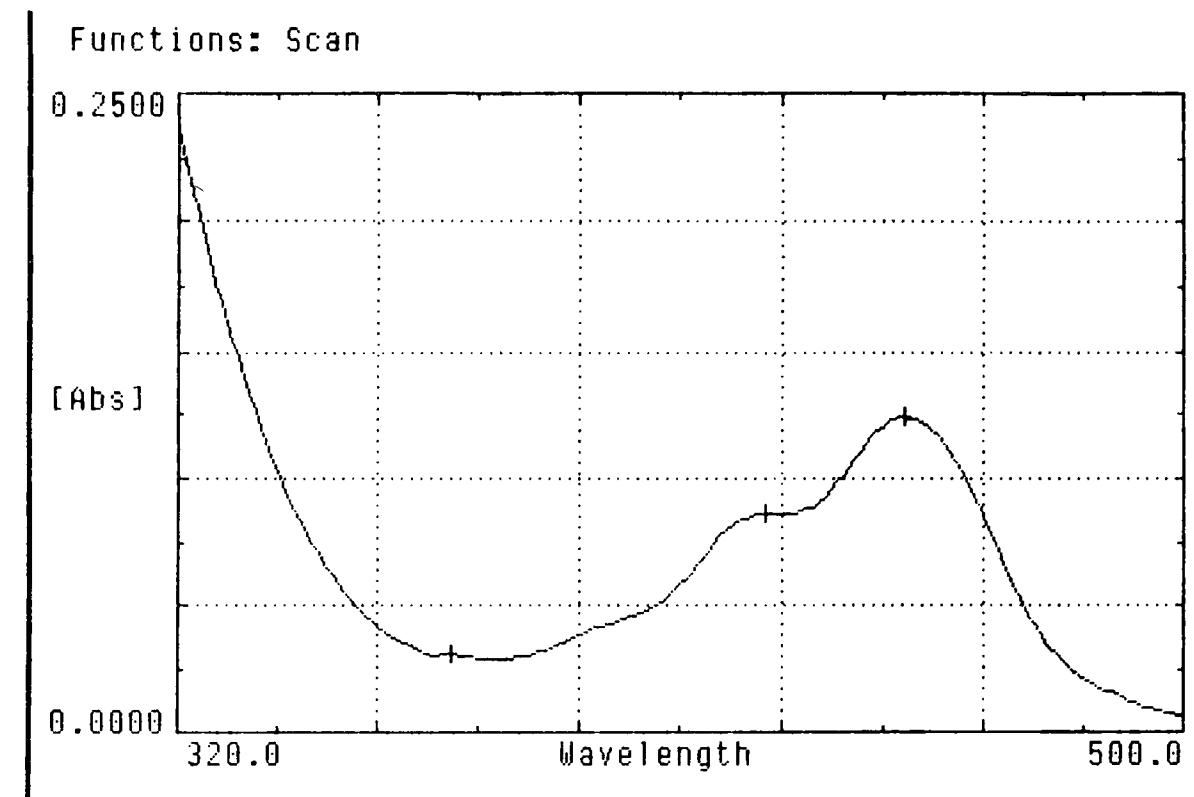
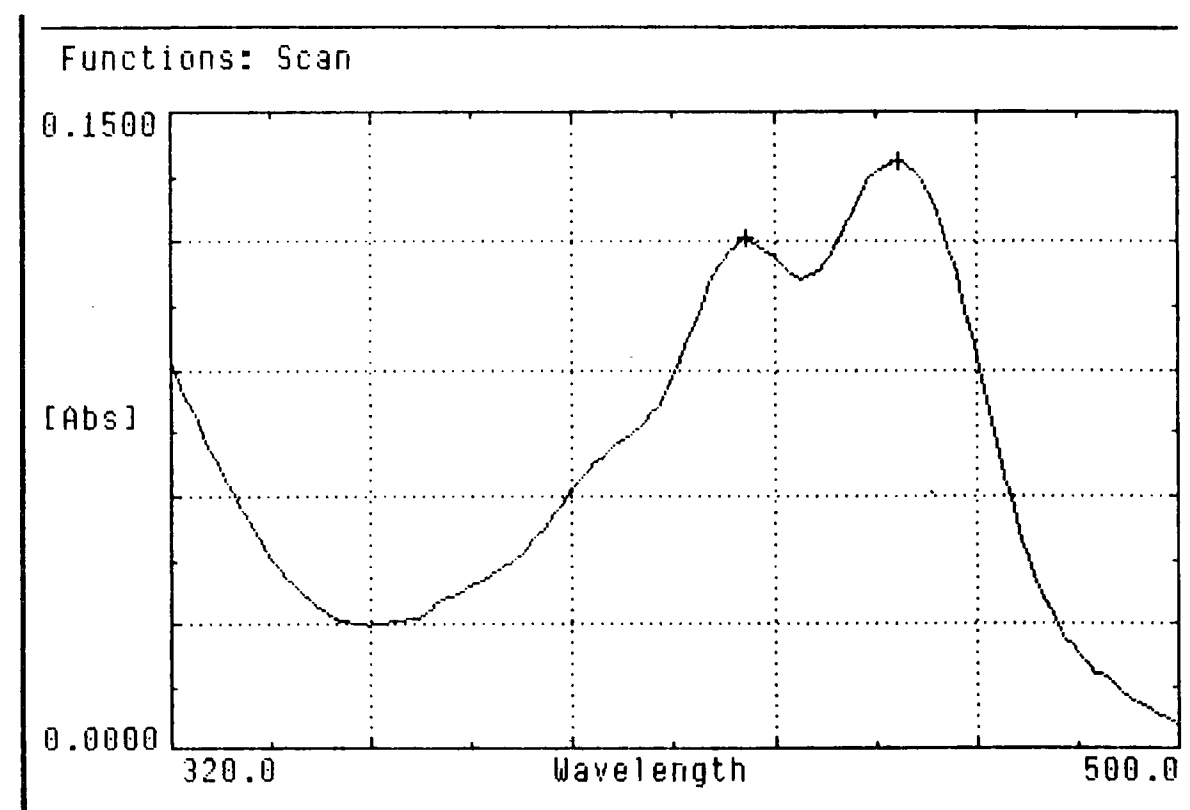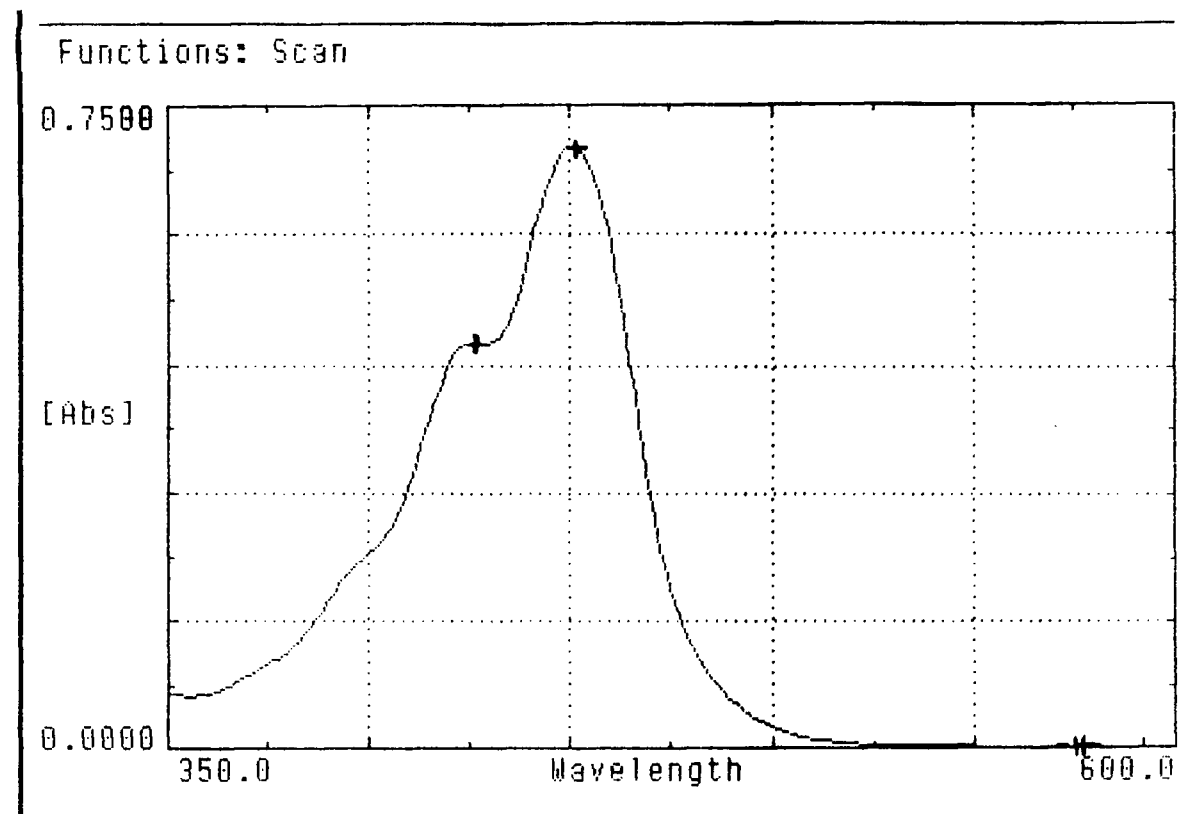b. Plants grown with their roots in 125g/l PEG for 16 days.

Fig. **6.3. Absorption spectra, in ethanol, of a crude diethyl ether extract of wheat roots.**

a. Plant grown with their roots in 125g/l PEG

b. Plants grown with their roots in 200g/l PEG.
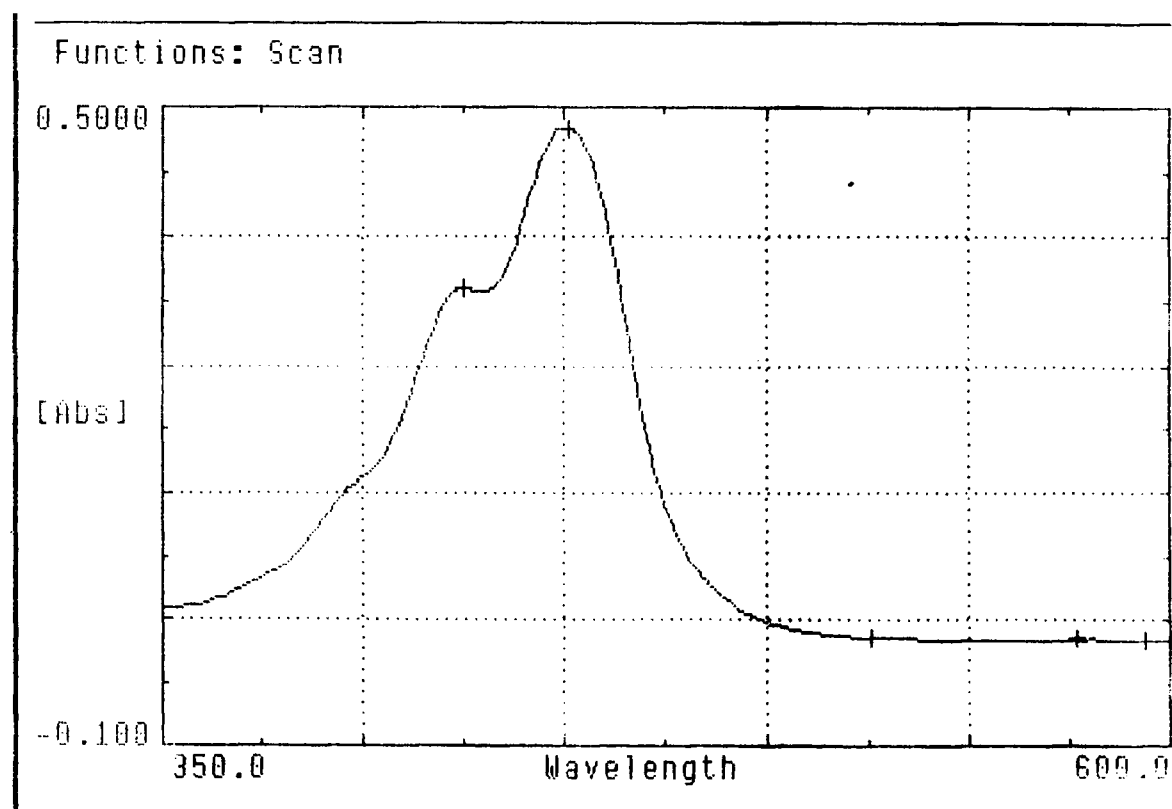
6.2 Characterization of the yellow pigment produced by roots in response to PEG-induced stress.

Purification and further characterization of the pigment was performed using Thin Layer Chromatography (TLC) and Mass Spectrometry (MS).

a. One-way TLC.

The solvent used here was diethyl ether on Silica plates impregnated with a fluorescence indicator. Development was until the solvent reached the plate top. Under these conditions the pigment migrated as a single spot and had an Rf value of 0.86 (Plate 6.2). No secondary spots were detected on the TLC plates indicating that the pigment sample was a relatively pure compound. Following removal of the plate from the chromatographic solvent and drying of the silica the pigment spot persisted unlike those for carotenoids from sunflower leaves.

Plate 6.2. Thin layer chromatography plate of wheat root pigment and sunflower leaf pigment extracts.
Samples of root pigment were loaded in the central positions with marker leaf pigment samples loaded on the outsides. TLC plates were developed using ether as a one-way solvent.
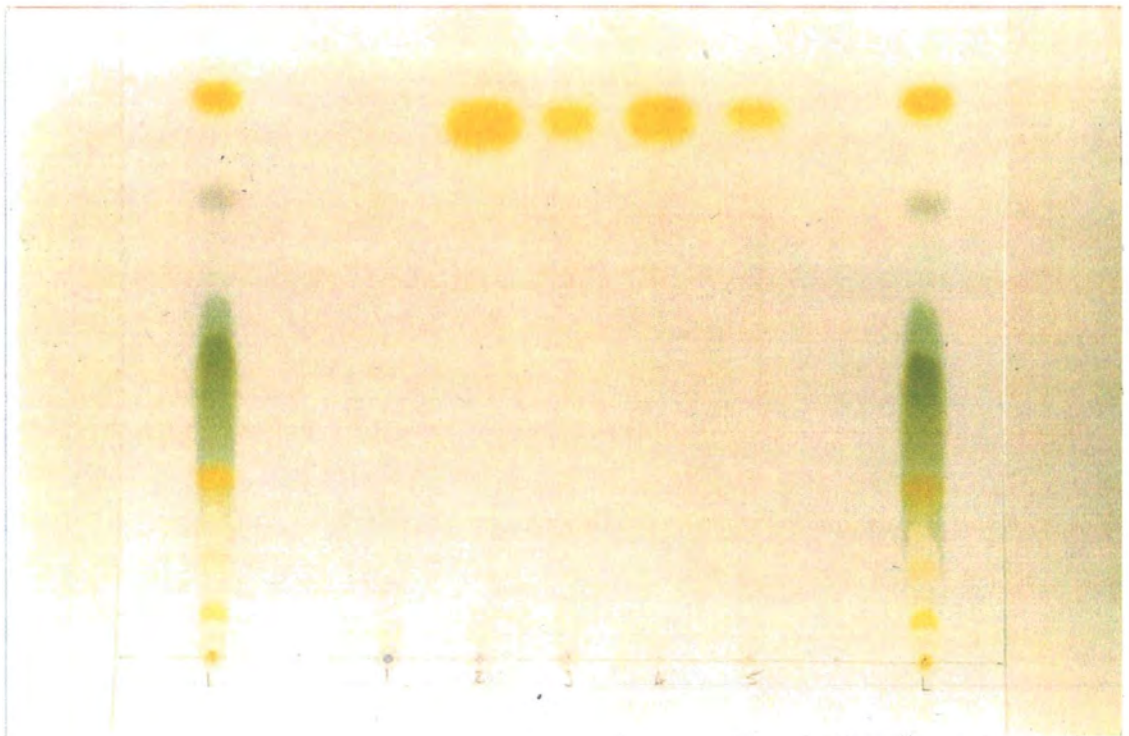
Fig 6.4 shows the absorption spectrum of leaf β-carotene extracted from sunflower plants. Here the spectral characteristics differ markdly from those of the pigment extracted from the roots. These observation indicate clearly that the root pigment was probably not a carotenoid. Exposure of plates to UV light of wavelengths 254, 300 and 330 nm did not result in any fluorescence of the root pigment samples.

Samples of the pigment removed from the plates and dissolved in ethanol gave absorption spectra which were almost identical to the crude samples indicating that the crude extracted material was of a high purity, Fig 6.5.

Further analysis of the pigment by TLC was performed using absolute methanol as the developing solvent which also gave a single spot essentially at the same Rf of 0.81 as for ether. With hexane : diethyl ether (9:1) again a single spot was apparent but with a Rf of 0.56. This pigment spot showed essentialy the same spectral characteristics, in ethanol, as the crude extract (Fig 6.6).

Fig. 6.4. Absorption spectra, in diethyl ether, of β-carotene from sunflower leaf tissues. Pigment was purified by one-way TLC using diethyl ether.

Functions: Scan

0.2500

[Abs]

0.0000

350.0   Wavelength   500.0

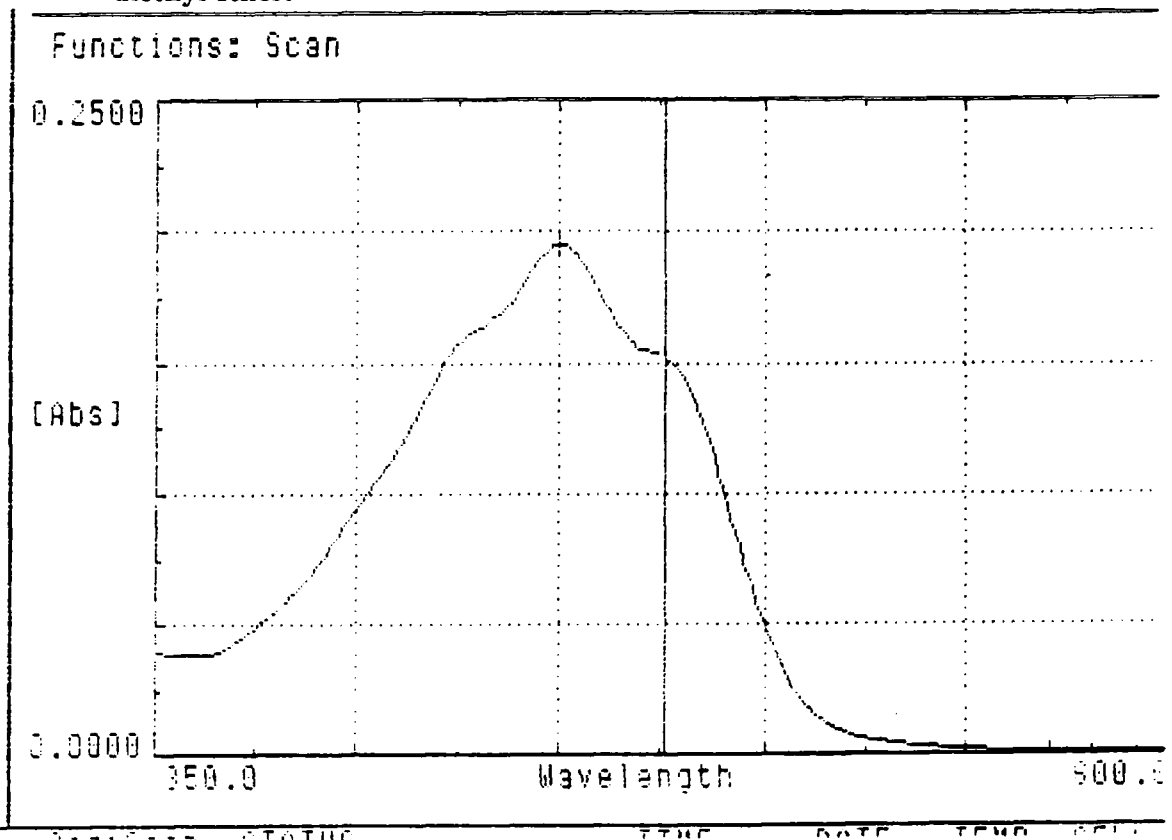**Fig. 6.5. Absorption spectra, in absolute ethanol, of wheat root pigment purified by one-way TLC using diethyl ether.**

Functions: Scan

0.7500

[Abs]

300   Wavelength   500
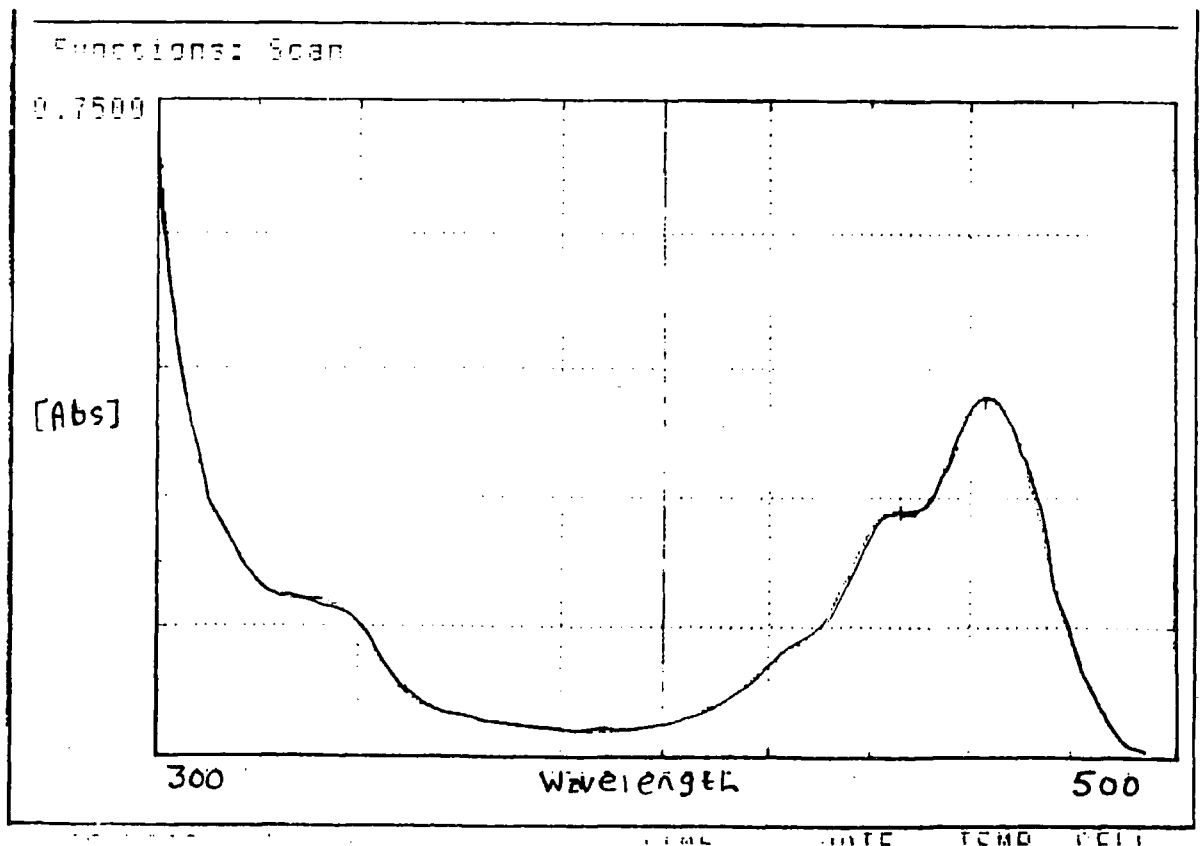
Fig. **6.6. Absorption spectrum, in absolute ethanol, of wheat root pigment purified by one-way TLC using hexane:diethyl ether (9:1)**
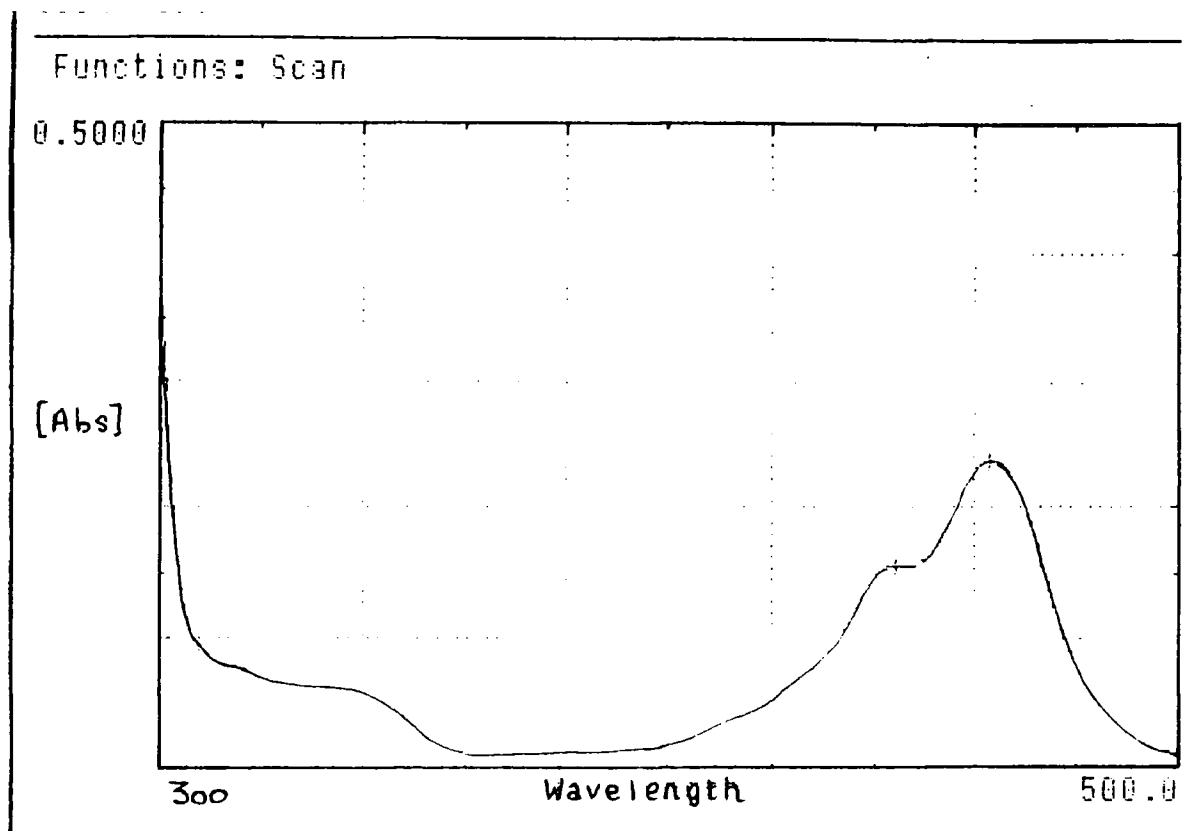
b. Two dimensional TLC

The first phase of this was with diethyl ether and the second phase with hexane : diethyl ether (9:1). Here again only one spot was obtained, the spectrum of which, however, had a slightly less absorption at lower wavelengths indicating the removal of some minor contaminants.

c Mass Spectrometry

In order to obtain information on the chemical nature of the pigment it was subjected to analysis by EI-Mass Spectrometry. This was performed at the University College of Wales, Aberystwyth. Two main putative mass ions were detected, M^+ 438 and M^+434 with a prominent m/z 57 ion characteristic of a tertiary butyl group. Full spectra are included in the Appendix.

Two putative structures for the chemical were suggested, Fig 6.7. Both of these compounds appear to be stilbenes. The M^+ 438 compound being 1,2 bis - (3,5 - ditert butyl - 4- hydroxyphenyl) ethane and the M^+434 being 3,3, 5,5- tetra tert butyl stilbene 4,4'quinone. However, the presence of the tertiary butyl group indicates a "non-biological" nature of the molecule. No further chemical definition analysis was carried out on the pigment.

Fig.   6.7. Putative chemical structures of the wheat root pigment assessed
by Mass Spectrometry after TLC purification
a. M⁻ 438. 1,2 bis-(3,5- ditert butyl -4- hydroxyphenyl) ethane.
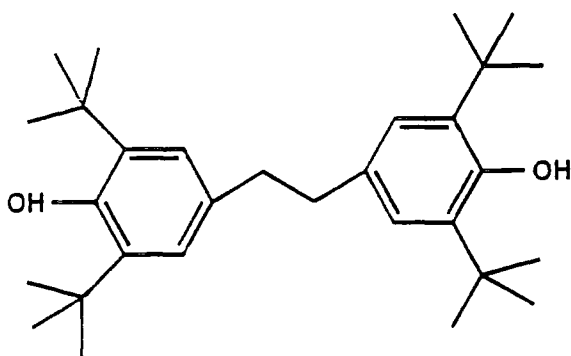
b. M⁻ 434. 3,3, 5,5- tetratert butyl stilbene 4,4′ quinone
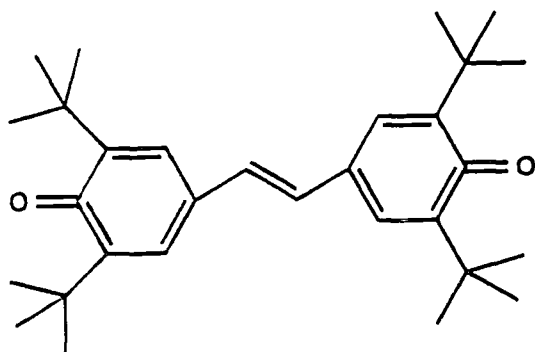
6.3 Biological significant of the pigment

The potential biological significance of this pigment was examined further by manipulation of the accumulation of it in roots.

To investigate if pigment accumulation was a response directly to water limitation wheat plants were grown with their roots in Mannitol and Sorbitol (125g/l). As has been shown in the previous Chapter these treatments stunted the plants and had an effect on the stomatal patterns. However, they did not result in the accumulation of the pigment, Fig 6.8. This indicates a specific response of the roots to PEG.

The effect of light and dark on development of the pigment was tested by maintaining seedlings with their roots in (125g/l) PEG for 14 days in a light growth room or in a dark growth chamber. At the end of the incubation the roots were extracted as before with ether and examined for the presence of the pigment. Fig 6.9 shows that the pigment was present in both light and dark grown materials. No pigment accumulation was noted in the control under either treatment. In order to test the persistence of the pigment, and any potential biological significance in the tissues, a series of transfer experiments were set up. Plants were grown in the light with their roots in nutrient solution supplemented with 125g/l or 200g/l PEG for 14 days over which time the roots accumulated the pigment.

Fig. **6.8. Absorption spectra, in diethyl ether, of a crude diethyl ether extract of wheat roots.**

a. Plants grown with their roots in 125g/l Mannitol for 10 days.
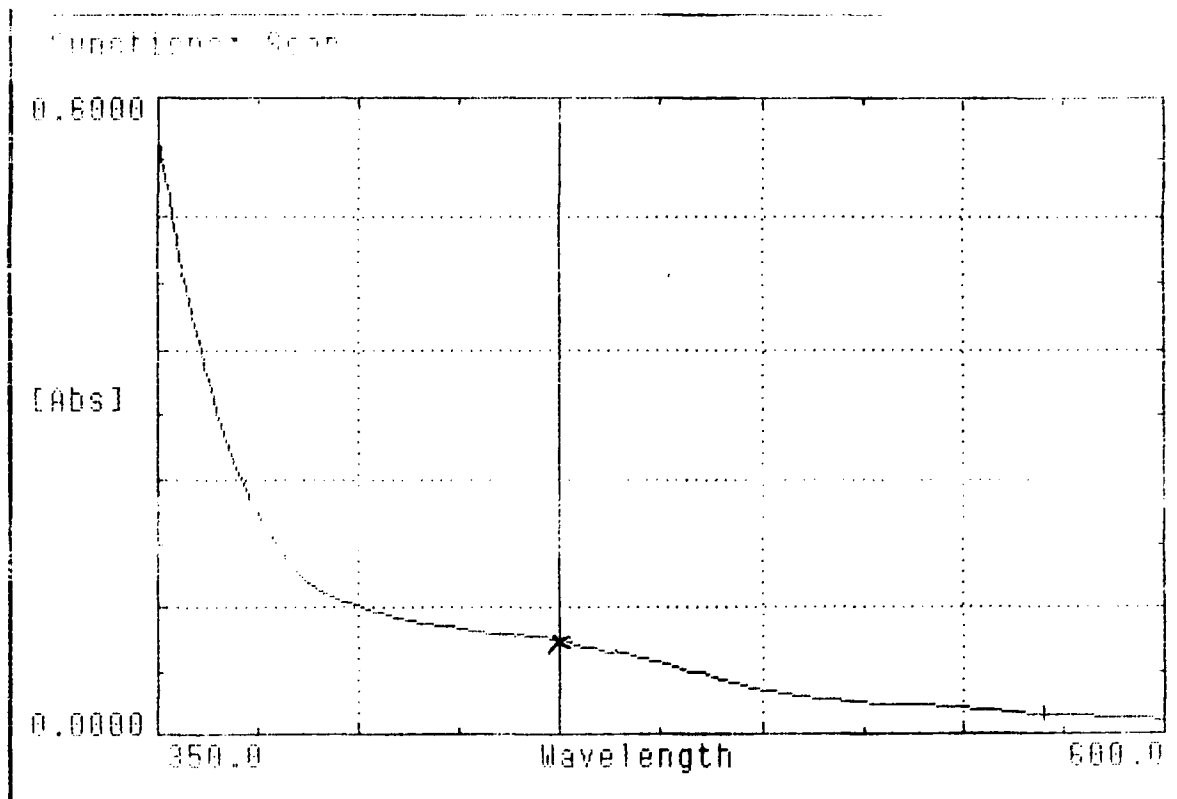
b. Plants grown with their roots in 125g/l Sorbitol for 10 days.
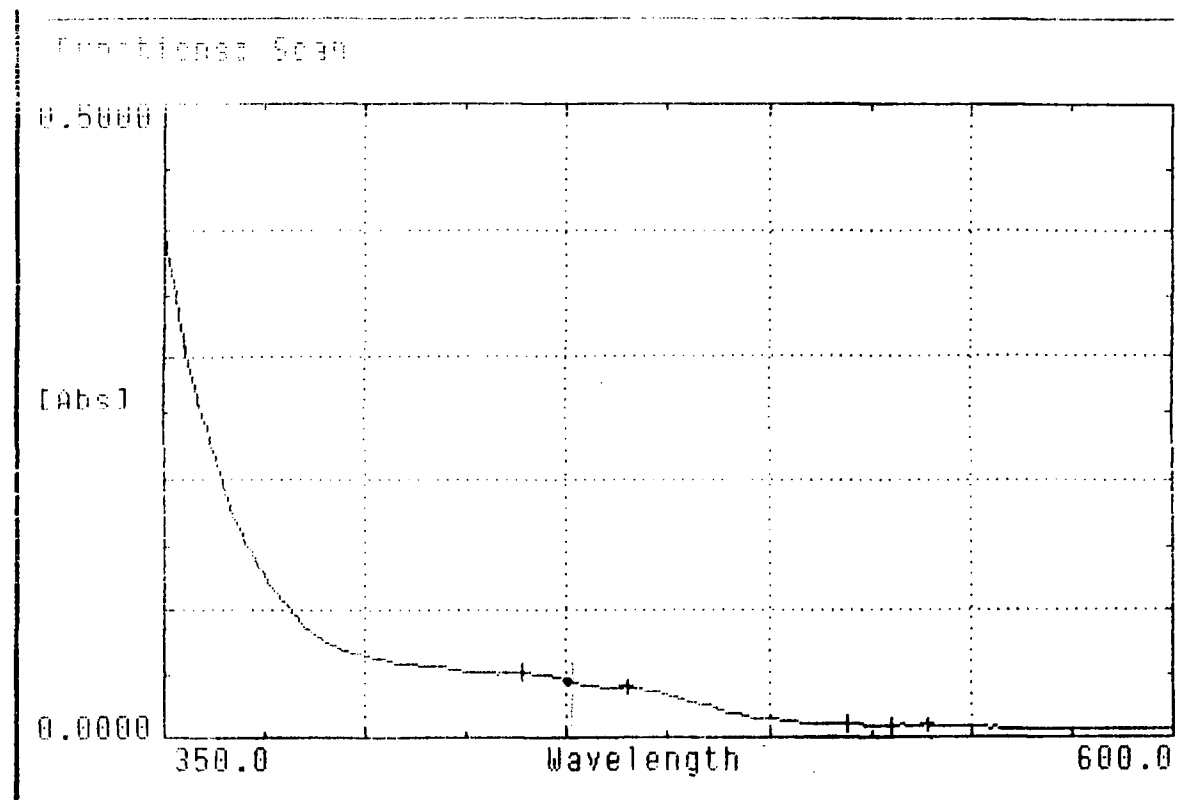
Fig. **6.9. Absorption spectra, in diethyl ether, of an crude diethyl ether extract of wheat roots.**

a. Plants grown with their roots in 125g/l PEG for 14 days in the light.
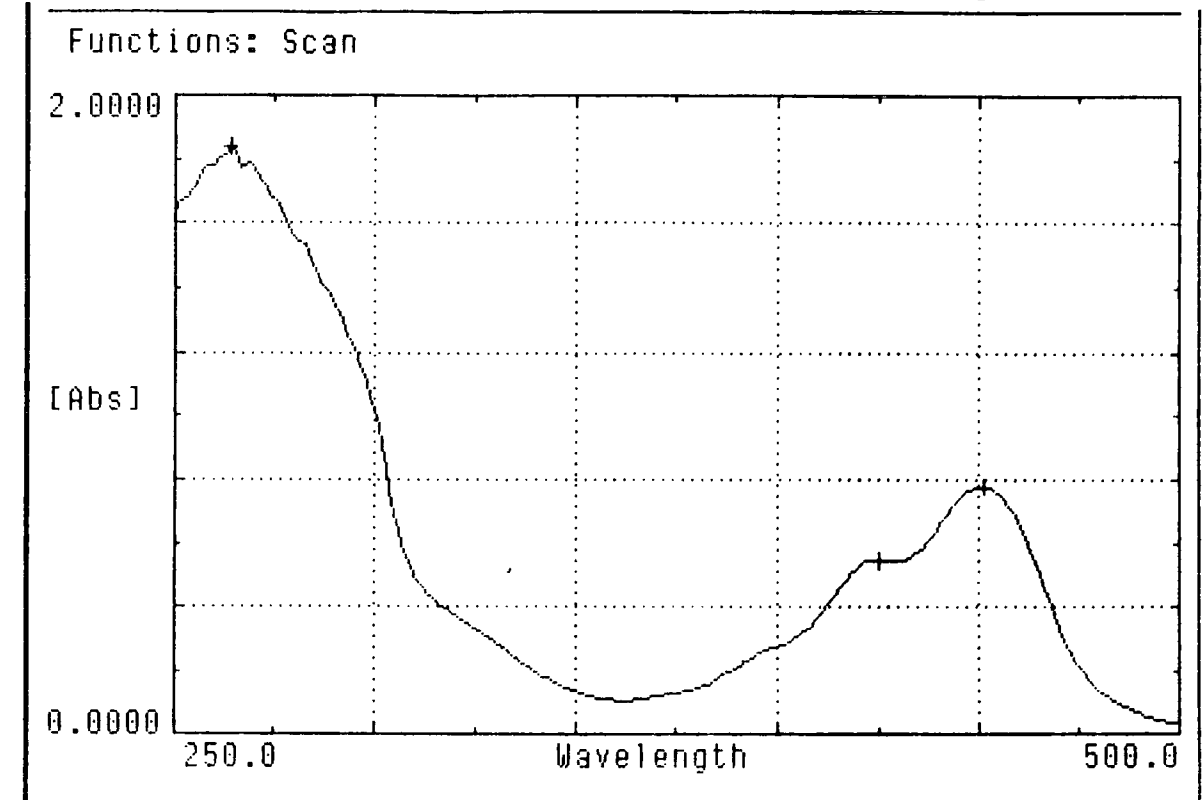
b. Plants grown with their roots in 125g/l PEG for 14 days in the dark.
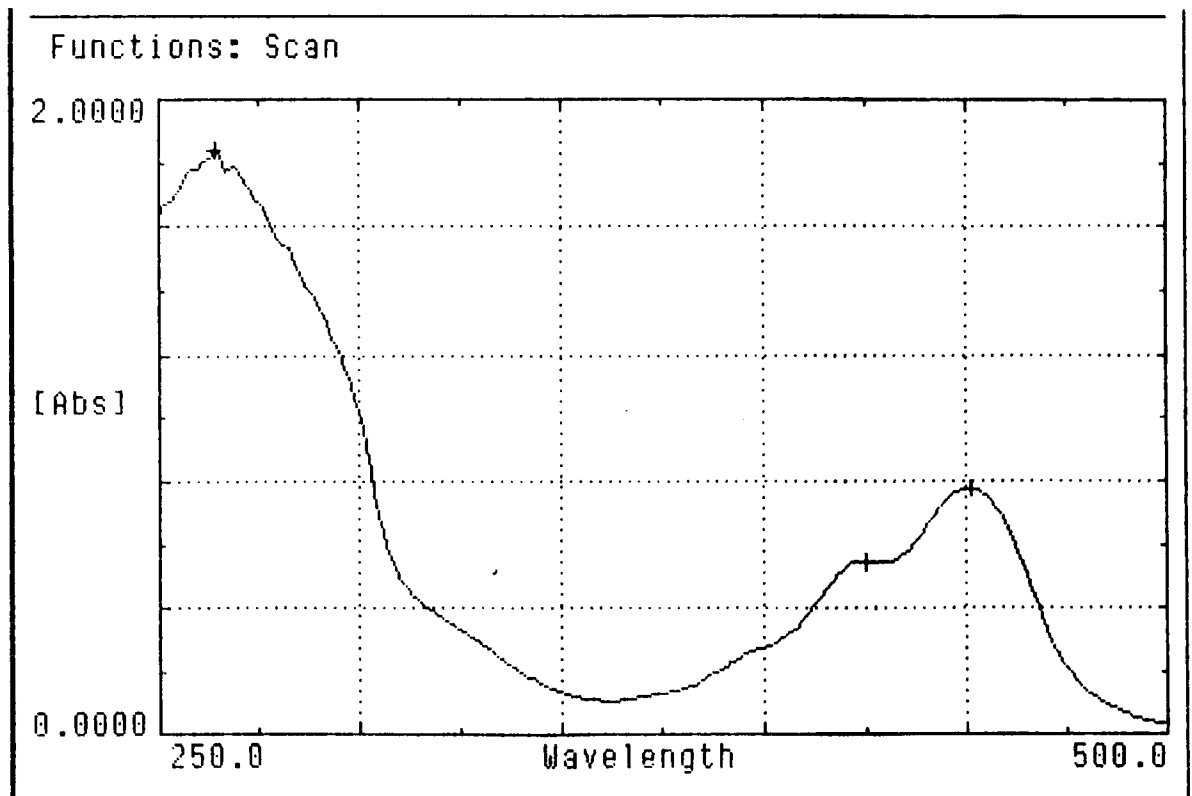
Batches of plants were then removed from the PEG treatments and were transferred for growth in nutrient solution only. At end of 10 days the roots were weighed and extracted for the pigment. Fig 6.10 and 6.11 show that the amount of pigment present, as measured by the peak height at 450 nm in relation to weight, was 1 in 125g/l and 1.3 in 200g/l prior to transfer. At the end of the second incubation the level were down to 0.23 and 0.44 respectively.

This indicates that some loss or degradation of the pigment took place in the living root system indicating some potential biological properties of it. No evidence of leaching of the pigment into the culture solution was apparent but it should be noted that the dilution factor would be very high.

It was also noted that new roots initiated in the second incubation phase i.e. after transfer to nutrient solution did not accumulate the pigment. It appears that roots must be in contact with the PEG in order to accumulate the pigment Also these appears to be no transfer of the pigment within the root system as a whole. This observation was further strengthened by the experiments involving split root incubations (Chapter 4, 4.10). Here half root system, where both were in PEG, developed the pigment. However, where one half was in PEG and the other in nutrient solution pigment accumulate only in those roots in contact with PEG.

Fig. **6.10. Absorption spectra, in diethyl ether, of an crude diethyl ether extract of wheat roots**
a. Pigment accumulated in 125g/l PEG after 14 days.
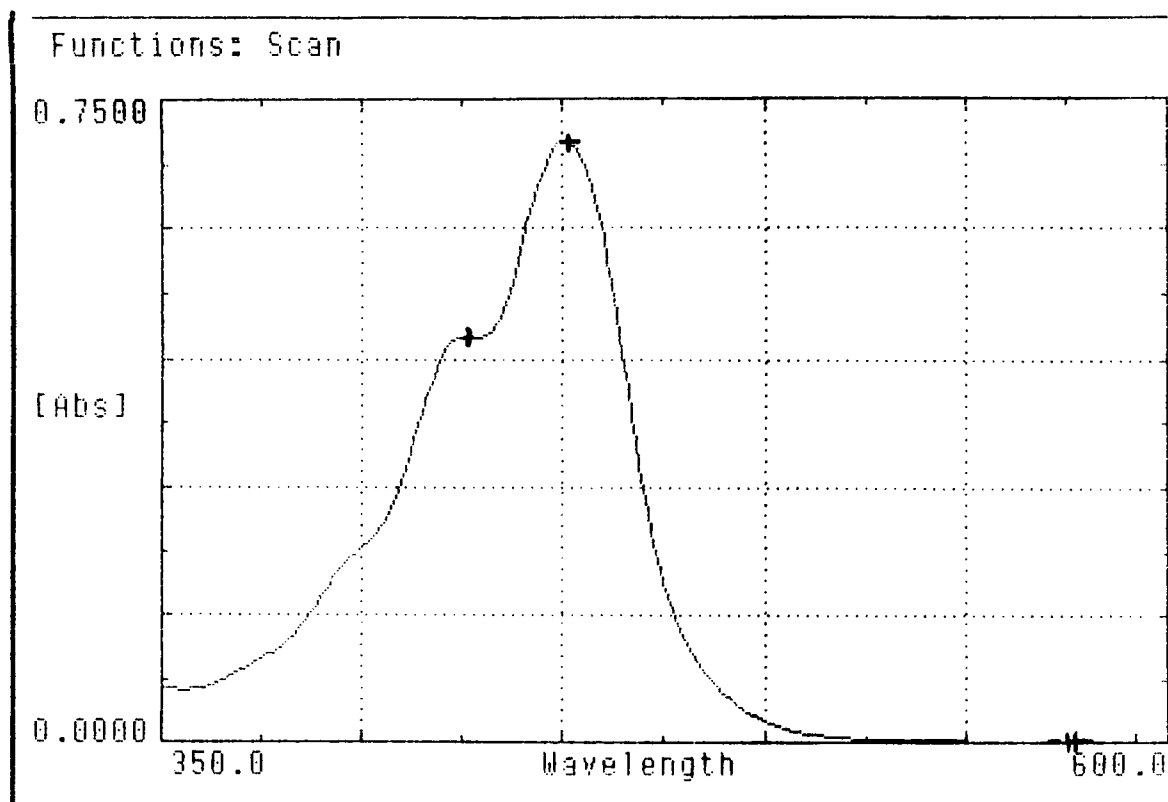
b. Pigment present in roots after 10 days incubated in nutrient solution following a previous incubation for 14 days in 125g/l PEG.
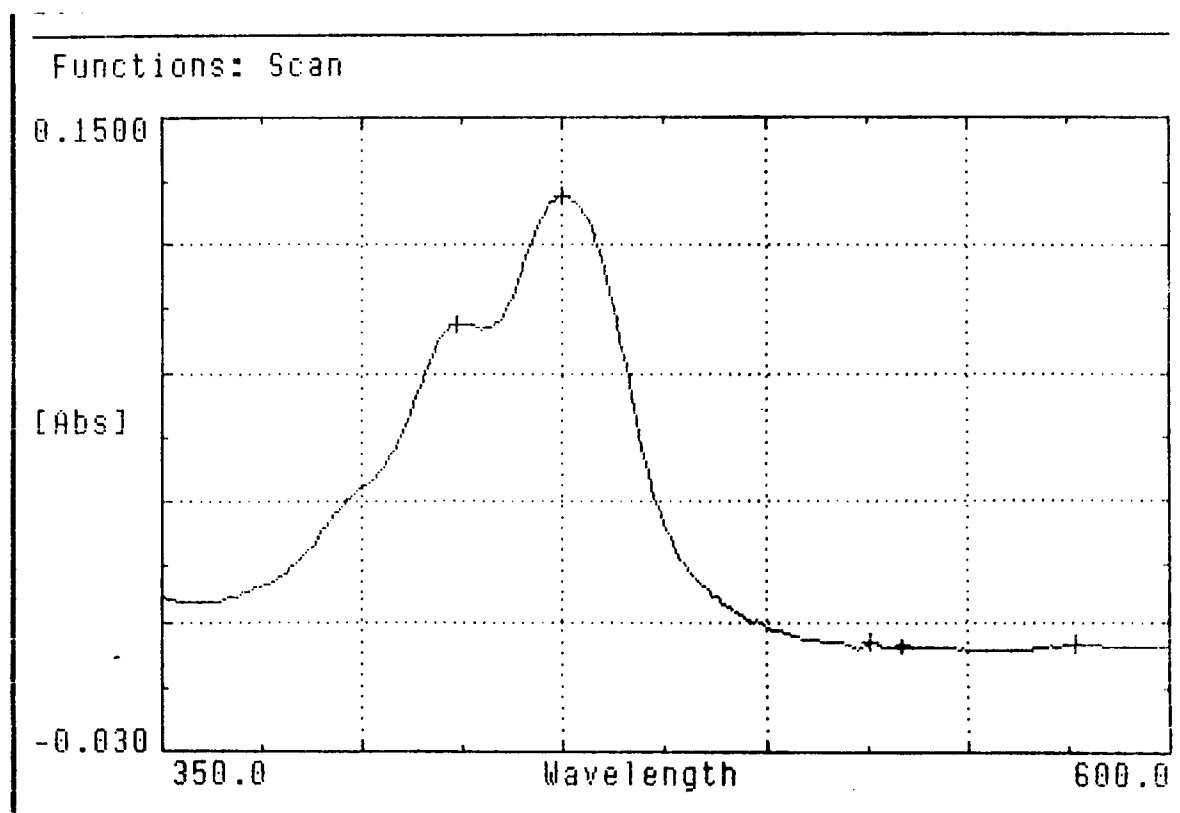
Fig. 6.11 Absorption spectra, in diethyl ether, of an crude diethyl ether extract of wheat roots.
a. Pigment accumulated in 200g/l PEG after 14 days.
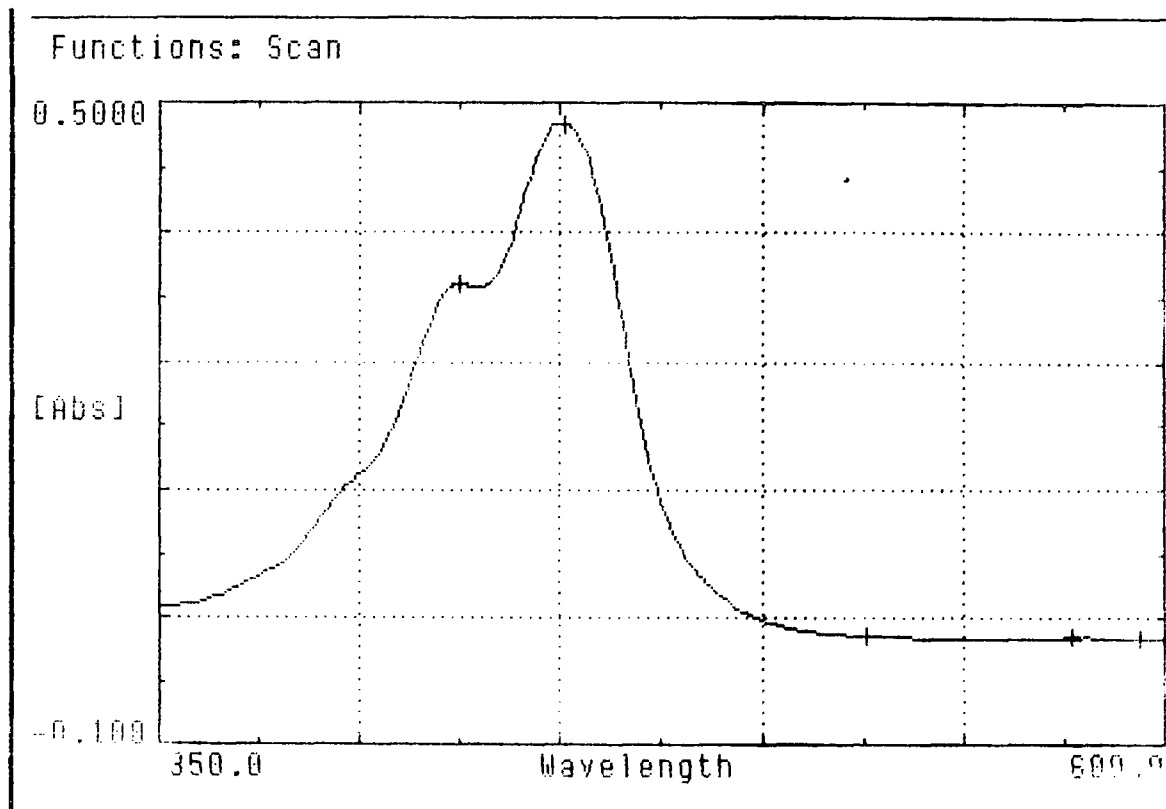
b. Pigment present in roots after 10 incubation in nutrient solution following a previous incubation for 14 days in 200g/l PEG.
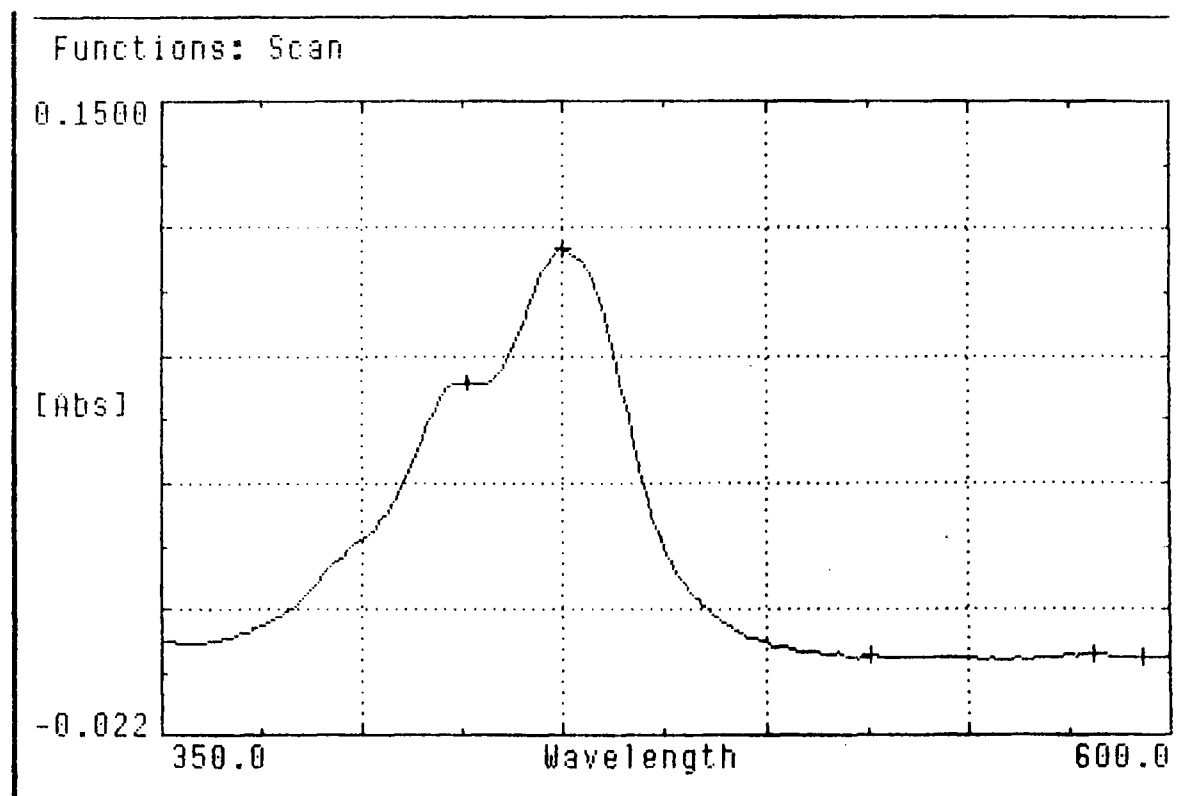
In order to assess the presence of this pigment in other plant roots under the same treatment, an investigation was performed using sorghum and maize. Seedlings were grown in nutrient solution with 125g/l PEG in the light growth room, as for wheat. At the end of the incubation period of 14 days the roots were harvested and extracted for the pigment using diethyl ether. Pigmentation was found and the spectral analyses indicated that it was the same pigment as found in wheat giving peak maxima at 424 nm & 449 nm, Fig 6.12 and 6.13. Here, however, a greater contamination at the lower wavelengths was seen compared with wheat.

ABA and NaCl treatments had an effect on wheat plant growth and stomatal patterns (previous Chapter), but they did not lead to a production of the pigment in the roots.

A microscopical analysis was performed to try and locate the pigment within the tissues. Section of pigmented roots were observed under normal light and UV microscopy. No consistent pigmentation patterns were observed. A yellowing of the root cell walls was observed but no distinct accumulation of pigment was seen in the cytoplasm.

Towards the end of this investigation a small amount of 12-month-old solid PEG 6000 was obtained and it was noted that this had a yellow colouration. Extraction of a sample of this PEG with ethanol gave a yellow pigmentation.

Spectral analysis of this showed that it was probably identical to the pigment

extracted from roots treated with PEG, Fig 6.14.

Fig. 6.12. Absorption spectrum, in diethyl ether, of a crude diethyl ether extract of sorghum roots grown in 125g/l PEG for 14 days.
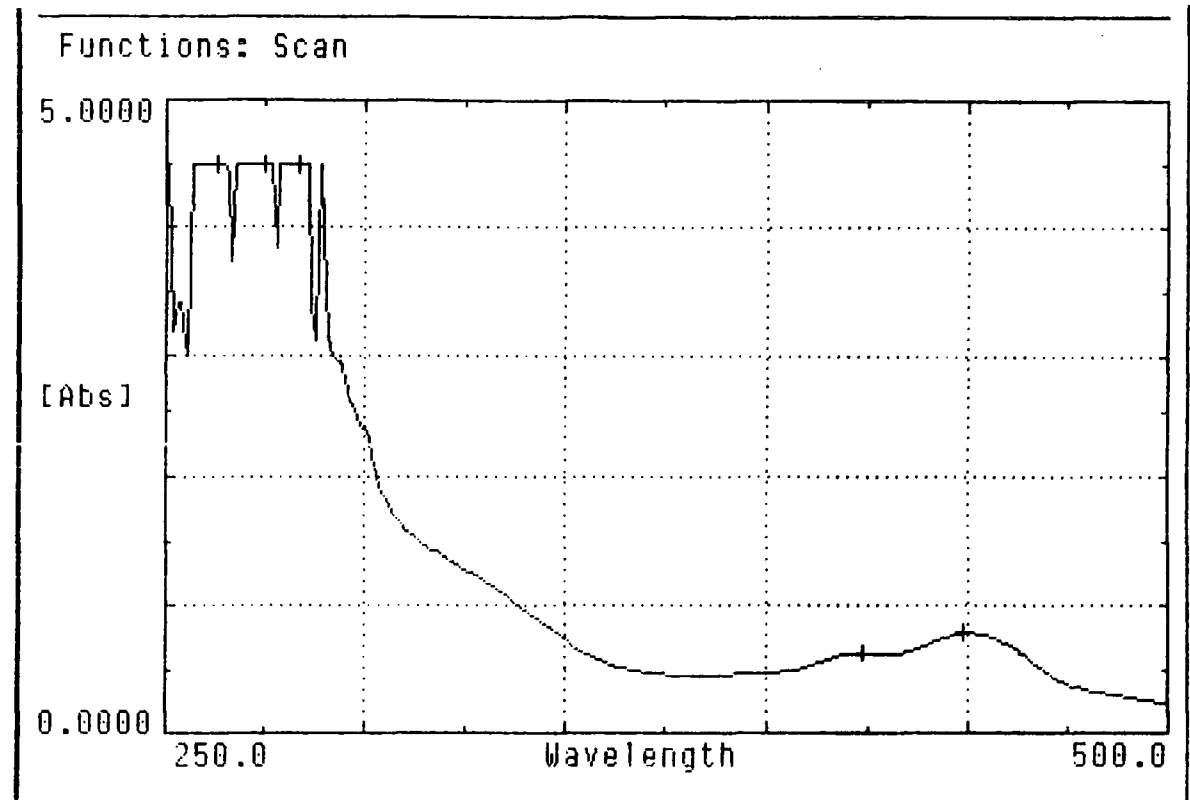
Fig. 6.13. Absorption spectrum, in diethyl ether, of a crude diethyl ether extract of maize roots grown in 125g/l PEG for 14 days.
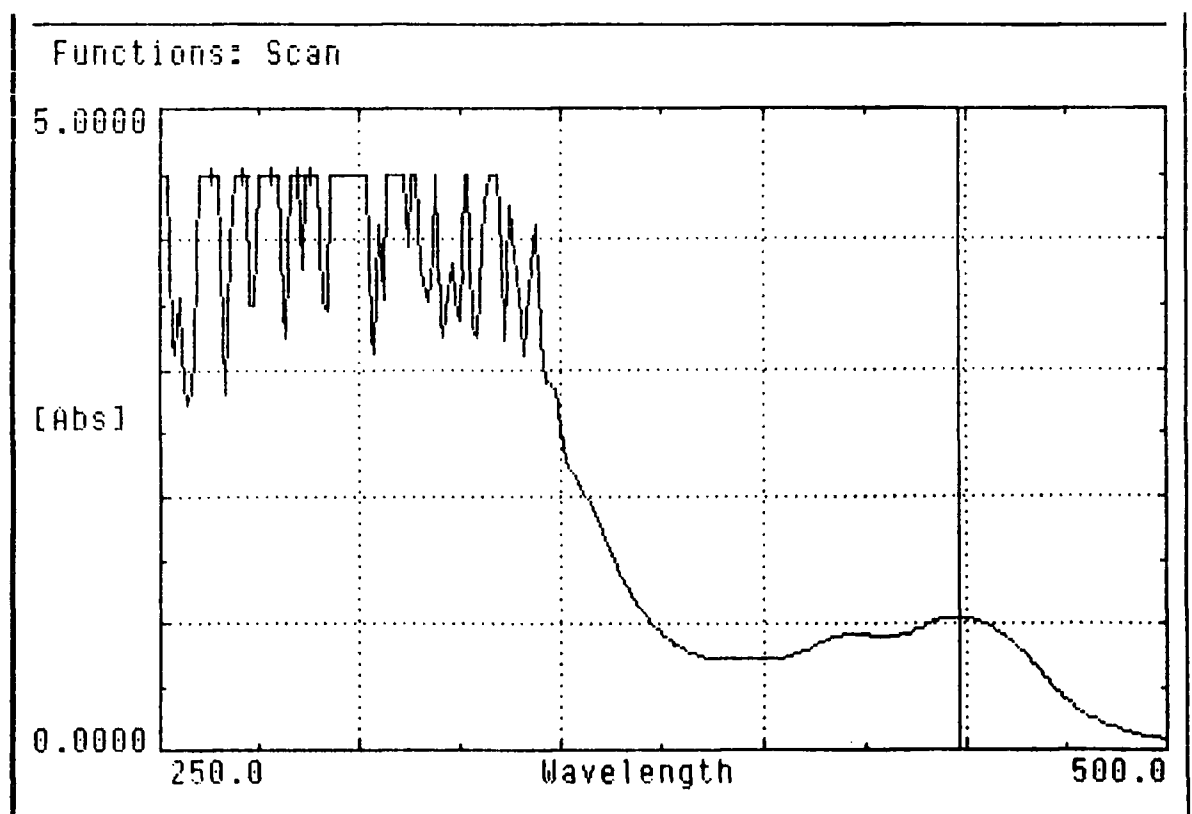
Fig. **6.14. Absorption spectrum, in ethanol, of an ethanol extraction of 12-month-old solid PEG.6000**
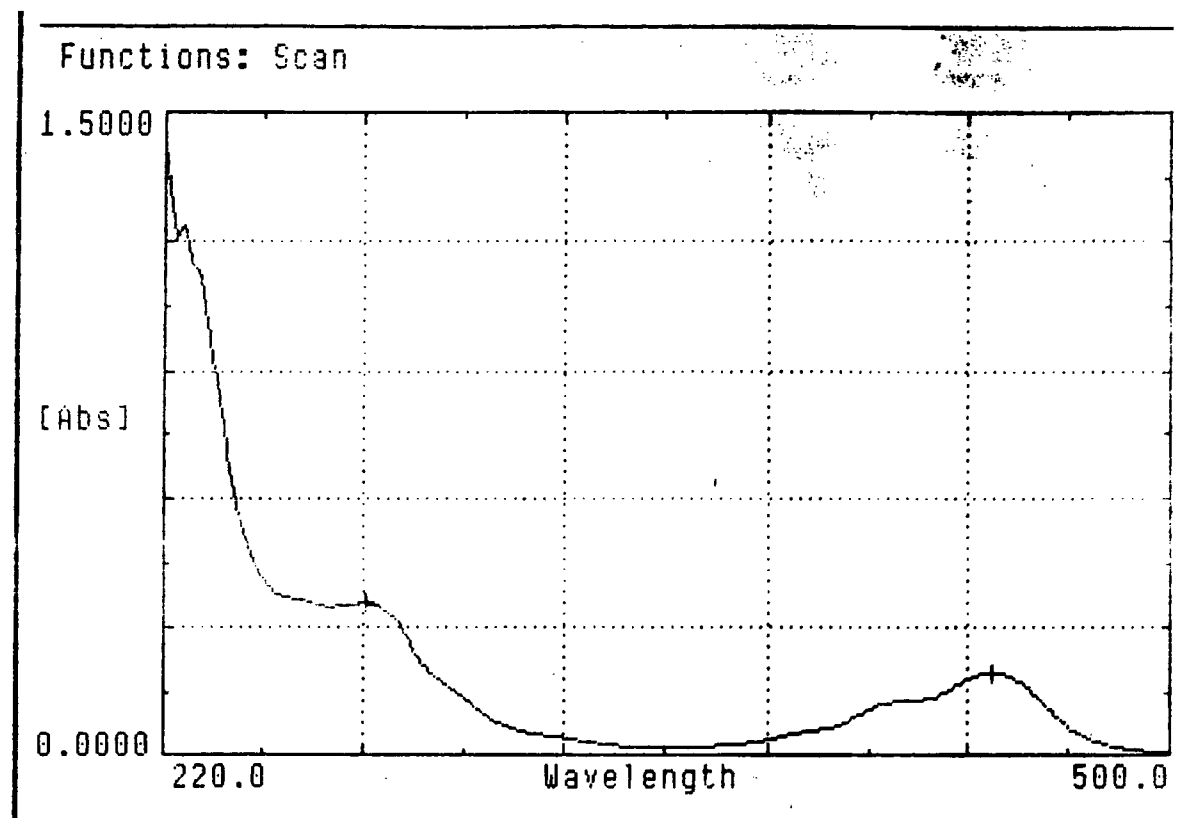
The implications are that the pigment which accumulated in the roots could be a product of PEG metabolism in the roots and/or the result of a reaction of the pure chemical to its environment. PEG could enter the roots system but not necessarily into the cells and therefore the pigment would accumulate in the cell wall. An alternative possibility is that the pigment could be present in PEG as a contamination/derivative at low concentrations which could be selectively accumulated by roots. New samples of PEG 6000 did not have a yellow colouration so it is possible that some degradation/derivatisation occured in the PEG during storage. However, pigmentation of roots was always seen when new, fresh and uncoloured PEG was used for inculation studies as was done throughout all experiments. This again indicates a reaction between the plant and PEG.

CHAPTER 7
DISCUSSION

Effect of watering regimes on plant growth and stomatal patterns of wheat seedlings.

It is well known that water stress tends to reduce both primordium initiation (Nicholls and May 1963; Hussain and Aspinall 1970) and leaf area expansion (e.g. Maximov 1929) and that it also modifies the frequencies of the different types of epidermal cells (Slatyer 1967; McCree and Davies 1974) including the formation of trichomes (Yapp 1912). Development of water deficits leads to a wide range of responses by plants (Hsiao 1973) affecting photosynthesis, respiration, absorption of water and mineral elements, nitrogen metabolism and reproduction. It is generally accepted that a reduction in cell growth is one of the most discernible effects of water deficit. Thus the results in this study on growth were similar to those generally reported from previous work.

The results presented here from a study of wheat and sorghum show that it is difficult to generalise how plants will respond to a changing environment, in particular to water limitation. The responses observed are mostly species specific. According to Martin et al (1983) drought resistance is determined by a number of physiological and morphological characters. The control of water loss by the stomata is one characteristic which many

physiologists and plant breeders believe may provide a means of improving drought resistance (Munchow and Sinclair 1989). Reduction in the growth is amongst the earliest discernible effects of a deficit of water in a plant. There is, however, considerable controversy about the relative sensitivity of water shortage on the two immediate components of growth; cell division and cell expansion Clough and Milthorpe (1975). In wheat plants the reduction in the water available clearly reduced the growth of the leaves as shown in Chapter 3.(3.2.1). This illustrates that cellular growth appears to be sensitive to drought. In the light of the literature we can conclude that decreasing the external water potential by as little as -0.1 MPa results in a perceptible decrease in cellular growth Salisbury and Ross (1985). A number of possibilities therefore exist in the way in which water availability will control growth. One important effect could be on the water loss potential through the leaves. It has been shown that under water stress some species have increased cuticle thickness (Cutler et al 1980). Other studies have indicated that water availability can be controlled through stomata patterns (Rawson and Craven 1980; Ferris 1991).

Reduced stomatal frequency is one selection criterion for drought resistance and there is evidence that this parameter can be associated with increased drought tolerance in cereal seedlings (Martin et al 1983). However, the problem is more complex than this implies, because if reduced stomatal frequency is the only selection parameter utilized, then plants with lower

numbers of stomata tend to compensate by producing larger leaves and large stomata and their water efficiency remains the same (Martin *et al* 1983). Such results reveal how complicated plant breeding studies can be, although, they may open up new lines of inquiry, because it may be possible to reduce water use by selecting for low stomatal frequencies, coupled with selection of other parameters, such as smaller stomata, smaller leaves or low stomatal index which were characteristics shown during this study (Munchow and Sinclair 1989).

It is known that stomata differentiation generally continues until the leaf has reached between 10 to 50 % of its final size (Ticha 1982). For a given leaf, total number and frequency of stomata will increase during this period then the frequency will decline as the epidermal cells continue to expand. Any environmental factors which influence the plant during this critical stage have the potential to alter cellular patterns, in particular stomata.

In investigating change in cellular patterns e.g., in response to water limitation it is important that not just growth features are considered but also specific effects of environmental factors on cell development and initiation. Many studies have concentrated on stomatal density as measure of change but this is related to growth, (Woodward 1987; Mansfield *et al* 1990; Woodward and Bazzaz 1988). Many early studies failed to calculated the stomatal index which is related to development but Beerling and Chaloner (1992) correctly state that the stomatal index must be calculated if the effects of treatment

difference are to be conclusive. Woodward and Bazzaz (1988) found the stomatal index decreased with increasing partial pressure of atmospheric carbon dioxide up to about 34pa and Gaudillere and Mousseau (1989) and Woodward (1987) found that the stomatal index decreased with a decreasing mole fraction of carbon dioxide.

This study has shown that exposing wheat seedlings to water stress results in differential responses for stomatal index, stomatal density and epidermal cell densities. The responses were the same both for leaf sheaths and leaf blades. Stomatal index decreased in response to a deficiency in available water, whilst stomatal density and epidermal cell density increased significantly. The decrease in stomatal index could be due to the inhibition of stomata initiation or differention from stomatal initial cells. However, judging from the stomatal index values, water stress treatments consistently reduced the proportion of stomatal complexes in relation to total leaf epidermal cells.

These results strongly suggest that varying watering regimes may influence directly or indirectly plastic responses by the plants in which there is an alteration of stomatal production and development. This would then influence the ability of the plants to survive under water limitation.

The results recorded in this study are in agreement with other observations in a wide range of species, where it was observed that water limitation increased the number of stomata per unit area under water stress. Rawson and Craven (1980) studied *Helianthus annus* L. and recorded that

drought conditions led to an increase in stomatal density. Ciha and Brun (1975) found that field grown Soybeans had significantly greater stomatal densities than non water stressed plants. If cell size, and hence organ size, in any plants is reduced by loss of turgor during expansion, it seems logical that leaves will have a higher stomatal density than those grown in the absence of water stress. An increase in stomatal density with water stress may be the result of restriction of expansion. To explain a decrease in stomatal density it is necessary to conclude that there is a reduction in the formation of stomata as a result of inhibition of guard mother cell formation or the differentiation of these into guard cells. In addition exposure to water deficit may have a developmental effect on guard cell size. In this study epidermal cells density increased in wheat following exposure to watering regimes, thus the increase in leaf size was because of increased cell divisions rather than an increase in cell enlargement. At the same time the length of the guard cells, including the pore, decreased.

The results presented in this work, indicate that wheat seedlings show an decrease in stomatal index with a reduction in water availability. These results are in agreement with the findings of Ferris (1991) who also reported that stomatal index of a three species, a grass, Sesleria caerulea L, a herb, Plantago lanceolata L, and tree, Acer pseudoplatanus L. decreased in relation to water stress. This suggests that water stress is affecting cell differentiation in the young developing leaves and thus inhibiting stomatal initiation. Stomatal

density and epidermal cell density will change and therefore stomatal index will be affected.

The reduction in the formation of stomata and the apparent reciprocal increase in trichome production may be related to the conservation of water. The role of the trichomes, however, is not clear, but they would be expected to increase the leaf boundary layer resistance therefore reducing water loss. Increased boundary layer thickness must also increase resistance to CO_2 diffusion into the hair-covered leaves. This is only a small proportion of the leaf boundary layer resistance expected in the field, 0.2 to 0.8 s cm^{-1} for wheat, according to Jones (1976). Such change in the boundary layer resistance would have little effect on the overall water economy, but would lead to an improvement in the water use efficiency where plants were subjected to high temperatures and large humidity deficits (Jones 1976).

The evidence presented here suggests that water regimes may have an important role in determining the pattern of plant development at a specific cellular level.

Effects of water-stress on plant growth and stomatal patterns of Sorghum cultivar seedlings

In this study the responses of six cultivars of sorghum differed from those of wheat in respect to growth and stomatal development when subjected

to the same level of water limitation. Overall, sorghum cultivars were found to be much less sensitive to a change in water supply than wheat. All six cultivars appeared to grow as well as the controls when subjected to water limitation by being supplied with water on a 4-and 7-day basis Chapter 3.(3.3.1). It is probable that these plants, which normally grow in dry habitats, posses a more efficient absorbing system relative to their transpiring surface. The sorghum leaf surface has a well-defined waxy cuticle which is important in the reduction of water loss Jordan *et al* (1984). In addition the higher stomatal resistance recorded for sorghum compared with that of other plants also may have contributed to sorghum's ability to conserve water and withstand stress. This study, as well as others Kirkham *et al* (1985), demonstrates that sorghum has a lower water requirement for growth and survival.

Munchow and Sinclair (1989) studied epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* L Monench. They reported that the ability of a plant to survive severe water deficit depends on the ability to restrict water loss through the leaf epidermis after stomata reach a minimum aperture when, at this stage, the rate of water loss is regulated by epidermal conductance. They observed that epidermal conductance increased with increasing stomatal density. An increase in stomatal density under water stress is usually positively correlated with epidermal cell density. Thus variables influencing epidermal cells growth may affect stomatal density (Salisbury 1927 Woodward 1987).

As for wheat, the study reported here shows that stomatal density and epidermal cell density of all sorghum cultivars increased with increasing water limitation in both blades and sheaths. However, overall, the magnitude of response was higher than for wheat but there was some variation between sorghum cultivars Chapter 3.(3.2.2.2).

Usually, where drought influences stomatal density, it does so by affecting the expansion of leaf area, causing the stomata to be packed more densely through changes in the size of the epidermal cells (Ticha 1982). The anomaly is that in this study sorghum leaves did not decrease in absolute size at the same time as stomatal density increased. Similar observations to these for sorghum have been here reported for the effect of elevated carbon dioxide on four chalk grassland herbs (Ferris and Taylor 1994).

It is possible therefore that in order to increase stomatal density new epidermal cells and stomata are produced under stress conditions but within the same size of leaf. Alternatively the stomatal index could be increased in order to result in more stomata. A slight increase in stomatal index was noted in the cultivar Saudi Red which could, in part, explain the density increase. However, the response was not consistent in cultivars; Funk, Indian White, & Morgon 854 maintained the same stomatal index whereas in others Morgon 856 and Pioneer 8ss, it decreased. It appears therefore that increased overall cell number may have been the factor operating in sorghum in some cultivars whereas in others an increase in stomatal index could be a contributory factor.

Considering the stomatal index alone, and hence differentiation of guard cells, it appears that sorghum does not respond in the same way as wheat where water limitation does influence stomatal production. However, it is possible that the level of water limitation was not sufficient in sorghum to trigger a marked developmental response specifically related to guard cell production.

As in wheat the trichome index in sorghum increased in response to water limitation, however, there was no reciprocal change in stomatal index. In sorghum it appears that trichomes are not differentiated at the expense of stomata.

Quarrie (1983) suggested that differences in the response of different species to drought are probably determined by genetic difference in the capacity for ABA production. Individuals species or cultivar may express different phenotypic responses under varying watering regimes.

The differences in response between wheat and sorghum are interesting in the light of their normal growth habitats. Wheat responds to water limitation by reducing stomatal differentiation which in turn would reduce carbon dioxide uptake and hence photosynthesis and leaf growth. To counter the water loss there appears to be a switch in development where trichomes are produced at the expense of stomata and these assist by increasing boundary layer resistance. This developmental switch appears to be based on the differentiation of guard mother cells. In contrast, in sorghum growth was not reduced by water

limitation, this is reflected in the fact that the stomatal index remained more or less constant. The result will be a maintenance of carbon dioxide uptake but at the expense of water loss. To counter this water loss it appears that more trichomes are differentiated to increase boundary humidity under water limitation . These new trichomes could arise from guard mother cells which normally do not differentiate in the leaf but remain in an initial state. This aspect was not studied in this project.

Effect of PEG on plant growth and stomatal characteristics of wheat seedlings

Addition of PEG-6000 to the nutrient medium caused changes in wheat plant water relations very similar to those in dry soil as was indicated by the reduction in growth. PEG of higher molecular weight is considered to cause an inhibition of the pathway of water movement, reducing water absorption and causing desiccation of the plant and hence inhibiting growth.

Alternatively leaf growth and stomatal responses to drought may be regulated by non-hydraulic signals, which are produced in roots in drying soil and move to leaves via the xylem (Davies *et al* 1994). Similar effects are possible where PEG may influence root metabolism so initiating a signal to the leaves.

Leaf growth

When plants are subjected to drought stress this causes an extensive range of responses, almost every parameter investigated is changed by this treatment; provided the stress is long and strong enough (Hsiao 1973).

Leaf expansion processes and their responses to stress play an important role in whole plant carbon and water economies. For potted, drought-stressed plants, Boyer (1970) reported that leaf elongation rates were considerably more inhibited than net photosynthetic rates. The responses shown in this study by wheat grown under drought conditions include a reduction in the leaf growth rate obtained within a specified time. The inhibition of growth by PEG may be the result of a subsequent action of its effects on cell expansion and enlargement (Fitter & Hay 1983). There is good evidence to suggests that under water stress plants begin to produce ABA (Wright 1969; Jarvis and Mansfield 1981). It is known that in many plant species ABA has an inhibitory effect upon growth (Milborrow 1974). A build up of ABA can inhibit shoot growth, lead to stomatal closure, reduced transpiration and could also limit leaf expansion.

Relating these findings to the present studies, it is clear that at mild and high concentrations of PEG the leaf elongation rate was reduced. This finding is in agreement with those of Matsuda and Riazi (1981) who demonstrated that sudden exposure to osmotic solutions brings about an almost immediate

cessation of elongation. Acevedo *et al* (1971) and Hasio *et al* (1970) noted that leaf elongation in maize was sensitive to a slight reduction in water potential and ceased at - 0.7 to - 0.8 Mpa. The data reported for wheat (Chapter 4) are in accord with those reported by Boyer (1970), and support the opinion of Hsiao (1973) that significant components of growth reduction in water-stressed plants could be due to insufficient turgor for optimal growth.

Experiments were conducted to study the impact of mild and high PEG stress conditions on the final leaf length and to investigate the effect of PEG regimes on leaf emergence. The final leaf length was reduced and the emergence of the leaves was delayed. However, the emergence of the leaves treated at 200g/l PEG was markedly reduced. These results are in agreement with those reported by Jefferies (1989) who showed that leaf growth in field crops of potato was highly correlated to moisture deficit. Growth of cells is highly sensitive to water deficit and expansion very much depends on the water status of the tissue which acts as turgor pressure upon the softened cell walls. Therefore, even under mild stress when turgor pressure is reduced by only few bars there is a significant decrease in growth (Hsiao 1973).

Dry and fresh weights of root and shoot.

The two principal methods of growth measurement determine increases in either volume or weight. Volume (size) increases are often approximated by measuring expansion in only one or two directions, such as length, height,

width, diameter or area. Volume measured as a displacement of water, has the advantage in that it can be measured at different times. Weight increases are often determined by harvesting the entire plant or its parts and by weighing rapidly before too much water evaporates from it. This give us the fresh weight. Because of problems arising from variable water contents, many workers with interests in crop productivity prefer to use the increase in dry weight of a plant or plant part as a measure of its growth. This parameter expresses actual increase in non-aqueous components within the plant and these are a measure of cell production. In many instances the water relation of plants are analysed by measuring water content (i.e the difference between fresh wt and dry wt weights) and expressing this as a percentage of fresh or dry weight.

In addition to morphological effects of PEG on wheat seedlings, reduction in total dry weight was significant. This result suggested that PEG has an inhibitory effect on the basic metabolic pathways of photosynthesis and is in accord with Hsiao (1973) who noted that drought stress has a similar inhibitory effect in the metabolic pathways. The fresh weight of roots followed a similar pattern but the effect here could simply have been an inhibition of water uptake or one of reduced translocation of metabolites from the leaves.

The mechanism by which PEG reduces growth in plants is not clear. However, four mechanisms may be responsible :

1- PEG entered the leaf and decreased the osmotic potential of the xylem solution or in part of the leaf;

2- PEG interfered with plant metabolism;

3- PEG lowered the surface tension with the leaf affecting cell permeability and

4- PEG blocked the transpiration pathway.

Lawlor (1970) reported that the effect of PEG on growth, is osmotic and that it does not have toxic effects. Marshner *et al* (1965) suggested that the deleterious effects of PEG are caused by its accumulation in the apparent free space of roots. In this study, at the highest concentration PEG, 200g/l the fresh weight of shoot and roots were very much reduced in respect to controls. The most satisfactory explanation is that the PEG moves into the margins and then spreads, decreasing the permeability of the transpiration pathway and water supply to the evaporating surfaces. Dry weight, on the other hand, showed significant difference between controls and treated tissues for both shoots and roots. The significant decrease in dry weight of the treated tissues was shown under the all concentrations of PEG.

Stomatal density

Stomatal density is variable depending on the leaf environment during cell division and elongation (Meidner and Mansfield 1968). Water availability appears to be the principal factor, other than CO_2, which affects stomatal

density (Woodward 1987). Increasing water availability could reduced stomatal density by increasing epidermal cell expansion. Conversely any limitation of water will result in an increase in stomatal density. The stomatal density of wheat was significantly increased at every level of PEG treated(Chapter 4).

Zhang (1989) reported for rice that drought inhibited cell expansion resulting in a reduction of leaf area and correspondingly increased stomatal density. He also reported that leaf insertion point had an influence on stomatal density and the response to drought. The results reported here (Chapter 3) show that stomatal density and stomatal index of wheat decreased with increasing height of leaf insertion. These results are in agreement with Pazourek (1969), who reported that for *Hordeum distichon*.

In contrast, the total number of stomata per leaf was consistently reduced by both ABA and water stress (Ciha and Brun 1975). The results reported here (Chapter 4) show that there are large effects of PEG treatment and ABA on the stomatal characteristics of wheat. In both cases the density of stomata was increased. However, density is not necessarily a measure of stomatal differentiation; this process can be more easily visualised by the use of the stomatal index. However, all PEG treatments led to an increased in stomatal density, with an accompanied decrease in stomatal size and epidermal cell size. This results are in agreement with Rawson and Craven (1980), who reported the a similar result for Tobacco

Stomatal index

The proportion of stomata to epidermal cells was calculated, as proposed by Salisbury (1927), on wheat leaves grown in various concentrations of PEG. The results obtained showed straight forward decreases in stomatal index under all PEG concentrations. This indicates that, as was seen for water regime experiments, treatment with PEG results in a reduced differention of guard cells.

However, there was lack of plasticity once the stomatal patterns had been established as was seen in the experiments where the plants were removed from PEG and transferred to nutrient solution. Here there was no reversal of the trend of development. This indicates that a "carry-over" effect will operate on wheat plants following exposure to a period of water limitation.

The osmotic effect was further investigated using mannitol or Sorbitol in place of PEG. Here a similar effect was seen where growth and stomatal index were reduced. The effects of these two compounds was not as great as that of PEG at the same concentration and this probably reflects a difference in osmotic potential.

The results are in agreement with the findings of Michel (1970) who showed that PEG 6000 produced greater inhibition of cucumber elongation at the same value of osmotic potential than mannitol.

Effect of ABA on stomatal patterns in wheat seedlings

Abscisic acid treatment led to an increase in stomatal density similar to that found for water limitation. This result is in contrast to that reported by Ciha and Brun, (1975) who observed that the total number of stomata per leaf was consistently reduced by ABA. It would be expected that since ABA reduces cell growth that the density of stomata should increase the results shown in Chapter 4 are consistent with this. Of more significance is the fact that ABA results in a reduction of stomatal index similar to that brought about by water limitation. This indicates that ABA is having a differentiation effect on the leaf as well as one on the basis growth. The results also complementary to the observations of Davies *et al* (1994) who suggested that there may be non-hydraulic signals from roots to leaves in order to influence stomatal function. ABA could be this signal since it is known to move within the plant.

Another response to ABA was the increase in Trichome index, again similar to water limitation effects. This effect may have been a specific direct response to ABA on trichome development or an indirect response due to inhibition of stomata formation with a compensatory production of trichomes.

Split root system

In order to investigate further the nature of the stimulus from the root system to the leaves which could alter stomatal patterns use was made of a split

root experimentation system. Here half root systems were grown in combinations of nutrient solution or nutrient solution supplemented with 125g/l PEG. For this arrangement it was expected that the influence of water limitation as a hydraulic effect could be to some extent removed since the roots in nutrient solution alone should have been able to absorb enough water for the leaf development. Any effect of PEG then could be due to the intiation of a stimulus in the roots. The results obtained in this study (Chapter 4) showed that the leaves of seedlings with half of their root systems in PEG and half in nutrient solution appeared to have growth comparable to that of the control (both half root systems in nutrient solution). Leaf growth of seedlings with both root halves in PEG was reduced compared with the other two treatments. This indicates that sufficent water was entering the leaves for growth and so reducing any hydraulic effect of PEG.

The result presented in Chapter 4 show that the wheat seedlings, with roots under stress gave lower stomatal indices than the seedlings with half of their roots with stress and half without stress. This indicates that a split application of stress to roots of the same plant may result in an effect on the shoot but the effect is considerably less than the application of stress to both roots. However, root growth was affected by water limitation with little taking place in nutrient solution with 125 g/l PEG. The effect of PEG treatment as visible growth was confirmed by dry weight determinations. In all cases the root halves grown in PEG had significant lower dry weight than those grown in

nutrient solution alone. This indicates that under water limitation through PEG root halves act independently. PEG has a local effect an the root growth and appears not to be transported into the half of the root systems in nutrient solution alone. These results are in agreement with Raymond *et al* (1966) who reported similer effects in roots where PEG inhibited growth.

Stomatal density measurements showed similar patterns as previously for those plants grown totally in nutrient solution or PEG. The latter treatment resulted in a higher density. However, when half roots were grown in PEG then the stomatal density was not different from the nutrient solution only treatment.

When stomatal index was investigated between treatments it was noted that again the response was as for whole plant treatments. Both roots in PEG resulted in a lower stomatal index compared to those with both sets of roots in nutrient solution. Plants grown with half roots in PEG and half roots in nutrient solution, however, had leaves where the stomatal index was reduced slightly, but not significantly, from the nutrient solution-only treatment but was significantly higher than for the PEG-only treatment.

These result indicated that if a non-hydraulic effect was operating on stomatal differention its effect was small. These results are to some extent complementary to the findings of Gallardo *et al.* (1994) who, working with lupins, reported that when only a part of soil was stressed and adequate water was available in a wet zone, stomatal conductance was not affected. However, here they were examining stomatal function not development.

The distinction between a non-hydraulic effect and a hydraulic effect of PEG on stomatal development was not resolved in the experiments carried out in this study. However, the fact that ABA altered the stomatal index did indicate that water availability alone was not the only factor to have an influence.

Effect of NaCl on germination, growth and stomatal index of wheat.

Germination of seeds may be affected by salinity through osmotic or specific ion toxicity, or both. Wheat seeds germinated in low concentrations of salt showing that they were able to absorb water from these solutions of salt but higher concentrations changed the external media to hypertonic and retarded percentage germination. These results are similar to those reported by Macke *et al* (1971) for *Puccinellia nuttaliana*.

There was apparently a sharp osmotic boundary between 10 mM and 200 mM. At 10 mM there was little or no significant change in germination from the control, while at 200 mM the maximum tolerance was apparently reached.

The significantly higher shoot and root fresh and dry weights for seedlings in 10 mM solutions than in nutrient solution indicate that growth may be favoured by small increments of salinity. This growth response is similar to

that reported for halophytic species by Brownell (1965). A reduction of seedling growth was obtained with increased salinity beyond 10 mM.

The effect of 50 mM sodium chloride (as a non-toxic level which inhbits growth) on stomatal density was significant. Stomatal density increased at this concentration of NaCl compared with the controls (Table 4.16). Here the effect could have been one of NaCl limiting cell expansion. However, the effect of the same concentration on stomatal index in wheat seedlings was considerable. After the period of experimental growth the stomatal index of control seedlings was greater than those of treated seedlings. These results suggest that NaCl acts as a stress agent and may affect cell differentiation in the meristem and thus stomatal initiation. The implications are that salt stress can lead to leaf response which will limit water loss. Such a response would be important in plants which could become acclimated to salt stress where water loss effects could be reduced.

Effect of PEG on stomata density, stomatal index, mother cells density, guard mother cell index and epidermal cell density in coleoptiles of wheat

Coleoptiles have been show to develop stomata and appear to have a functional role. These have been implicated in tropic movements and water loss Mcintyre (1994). In this study it was noted that what appeared to be functional stomata were found associated with the tip region of the coleoptiles as indicated by shape and starch content (Chapter 5, plate 5.3). Those nearer the

base, in the mid section of the coleoptiles, had less starch and appeared to be closed indicating that they may no longer have been functional. However, such difference may be a reflection of maturation of the structures (Bunning and Biegert 1953). Coleoptile stomata must have a function in the growth of the structure and because of the simple nature of these stomata in coleoptiles they were used as a model system for development.

The cellular complexity of the coleoptiles is much lower than that of the leaf and hence individual stomata are more easily observed. However, some differences were noted between the response of the coleoptiles and leaves under the same PEG treatment. ABA treatment gave essentially the same effect in both tissues where index was decreased.

When coleoptiles of wheat were treated with a range of PEG concentrations reductions were seen in both stomatal density and guard mother cell stomatal index while there was no change in guard mother cell density and index under the same concentrations of PEG (Chapter 5). However, epidermal cell density increased significantly. The reduction in stomatal density in coleoptiles contrasted with the increase in leaf tissue under similar PEG treatments. The reason for this difference in response is not apparent.

Shortage of water produced characteristic deficiency symptoms, so coleoptiles experiencing drought conditions were unable to grow efficiently. New cells produced by cell division expand by taking up water, and so if water is in short supply this step in the growth of cells will be restricted resulting in

the reduction of coleoptile area, increased epidermal cells density and hence decreased the epidermal cells size.

Since production of guard mother cells in coleoptiles appeared not to be influenced by water status of the tissues it is concluded that control of stomatal number occurs at a stage between guard mother cell formation and guard cell differentiation.

Effect of PEG on stomatal density and stomatal index in wheat coleoptile in the presence and absence of roots

In all coleoptiles treated with PEG 125g/l as a water limitation agent, stomatal index decreased. However, appreciable decreases in the final percentage of stomatal density and stomatal index were seen in the intact seedlings ie caryopses with roots and with coleoptiles attached. It was observed that the presence of roots and caryopses played an important role in decreasing the final percentages of stomatal density and stomatal index. These results indicate that the root may be an essential component in the responses of coleoptiles to drought stress in terms of stomatal development patterns. However, a signal from the roots may also influence shoot growth. Inhibition of root expansion and elongation of coleoptiles was evident in PEG 125g/l because of its inhibition of cell expansion and enlargement.

The results recorded here are in contrast with the finding of Cramer and Bowman (1991) who working with maize seedlings reported that root system is not essential to the initial response to leaf elongation rate to salinity. As for

whole seedlings it is possible that roots produce signal compounds which influence cellular patterns in coleoptiles.

Effect of ABA on stomatal index and guard mother cell index in wheat coleoptiles

It has been reported in (Chapter 4) that ABA treatments led to reduction in the growth and alterations to stomatal patterns of wheat seedlings. However, a more detailed study of the effects of ABA on stomatal patterns was made using wheat coleoptiles. Results in Chapter 5 (5.3) indicate that in contrast to PEG, ABA treatment reduced both the guard mother cell index as well as the stomatal index in wheat coleoptiles. The results indicate that there was an effect on the potential to produce stomata rather than on the differention process from guard mother cell to stomata. Possibly by the inhibition of specific cell formation in leaf primodia. How this may be brought about was not addressed in this study.

Effect of light on stomatal density and growth of wheat coleoptiles

Light is probably more important in determining the course of stomata behaviour than any other environmental component (Meidner and Mansfield 1968). The quantity, quality and timing of irradiance can also substantially affect the growth of a plant. The R/FR ratio is thought to affect leaf growth and stomatal differentiation. Since light influences the functioning of stomata

the possiblity existed that it could also influence their development. To investigate this in coleoptiles the wheat caryopses were germinated in the dark with the result that some stomata were produced. Exposure of coleoptiles to light gave a slight decrease in stomatal density. This was in relation to a decrease in cell size which should have resulted in increase density. No data was available for the effect of light on the stomatal index.

It is apparent that water limitation has effects on stomatal patterns. Stomatal density changes are probably related to water uptake and cell expansion. However, the effects of water limitation on stomatal index indicate some effects in the differentiation proceses. This study showed that hydraulic effects were apparent but non-hydraulic effects could also have been operating, possibly through ABA as a signal factor. Similar effects have been seen for plants grown in soil and deprived of water as for those grown in hydroponic culture with PEG. However, in this study, using PEG, some non-developmental effects of the compound were noted in the roots of wheat. These effects included the appearence of pigmentation within the root tissues.

Root pigmentation in wheat

During the course of the study on stomatal production it was noted that wheat roots under PEG treatments were yellow, with the intensity of the yellow colour unrelated to the amount of PEG applied. Roots grown in nutrient

solution with out PEG did not show the yellow colouration. The appearence of this pigmentation was specific to PEG treatment, it did not appear when roots were grown in sorbitol or mannitol when these were used to limit water availablity.

The pigment accumulation appeared to be a specific metabolic event and not due to a concentration effect in roots shortened by water limitation. This was indicated by the lack of effect of mannitol and sorbitol which also shortened the roots. Pigment accumulation was also seen in sorghum and maize under PEG treatment. Pigmentation was not seen in roots treated with ABA which indicates again a specific response to PEG and not to the potential signal produced in the plant in response to water limitation. Further evidence that the pigment was produced as a consequence of biological activity was obtained from experiments where plants were transfered from PEG back to nutrient solution alone. Here the pigment levels declined although they did not disappear completly from the old roots. New roots initiated after transfer did not contain the pigment.

Pigmentation of root systems of plants under water limitation has been reported by other workers although they did not report any characterization of the compound (s) involved. Zgurouskaya (1955) observed this effect in plants grown in soil whereas Raymond *et al* (1966) noted pigmentation development in hydroponic culture in the presence of PEG. In this study reported here (Chapter 6) attempts were made to isolate, purify and identify the pigment (s).

It was initially suspected that the root pigment was of a carotenoid nature since these compounds are known to act as potential precursors for ABA (Neill *et al* 1984). The analysis of this pigment by TLC, spectral analysis and Mass Spectrometry, however, showed that it was not a carotenoid.

Initial characterization by Mass Spectrometry, indicated two potential structures for the pigment:

1,2 bis-(3,5-ditertbutyl-4-hydroxyphenyl) ethane.

3,3,5,5-Tetratertbutylstilbene-4,4-quinone.

Both of these compounds appear to be stilbenes although the presence of tetratert butyl group does indicate a "non-biological" nature of the molecule. Stilbenes are phenolic compounds which contain two benzene rings separated by an ethane or ethene bridge. These compounds are known to be secondary metabolites and they are collectively disignated as the stilbenoids (Gorham 1980). They have been isolated from higher plants. Their main physiological roles are not yet fully known but include actions as phytoalexins and growth regulators. This latter property may be of significance here given that plant growth is reduced by PEG, this inhibition may be through a chemical effect as well as through water limitation.

Extraction of commercial one-year-old PEG-6000 also yielded a yellow pigment which gave the same spectrum as the pigment produced in the roots. No mass spectrometry analysis was performed on these compound. It is possible that the same metabolite of PEG is produced both by roots and during

ageing of PEG. However, the fact that the pigment was found in roots when it was not apparent in the initial PEG material indicated a specific response within the plant to the chemical.

These results here indicate that care must be taken in the interpretation of water limitation experiment using PEG. In addition to limiting water supply to the plants this compound may also have secondary metabolic effects which are yet to be characterised e.g., direct limitation of growth. It is known, however, that PEG 200 can enter root systems (Lawlor 1970) although PEG 6000 may be too large to do this hence PEG 200 could be subjected to root metabolism / detoxification in cells. PEG 6000 could, however, be metabolied in the cell wall without it entering the protoplast.

Further investigations here could include the characterisation of any direct growth inhibitory effects of the pigment. To do this, however, large scale extraction and purification steps would need to be introduced.

CONCLUSIONS

1)   Seedlings of wheat and a number of sorghum varieties grown under water limitation conditions showed a considerable variation in the leaf growth, epidermal cell density, stomatal density and stomatal indices within and between species. Both species showed significantly increased leaf epidermal cell densities and stomatal densities. Significant decreases in the stomatal index with increasing water limitation were found in wheat leaves. This patterns also occurred in sorghum cultivars Morgon 856 and Pioneer 8ss under 7 days watering regimes but not in the other cultivars examined. The results of this study strongly suggest the existence of a plastic cellular response in both species to varying watering regimes. Trichome indices increased significantly in the two species under watering regimes which may be related to water loss reduction.

2)   Seedlings of wheat grown with PEG added to hydroponic growth medium showed marked reductions in their leaf growth as well as their dry and fresh weights. Stomatal density increased significantly but decreased stomatal indices were noted. Guard cell length and width decreased significantly. Similar responses for stomatal index were also seen in seedlings when Mannitol and Sorbitol were added to the growth medium indicating an effect of water limitation on stomatal differentiation.

3)   Plants grown under different concentrations of Absicic acid showed a significant decrease in stomatal index and an increase in trichome index.

4)   Plants grown under a range of NaCl concentrations showed delayed germination with increasing NaCl concentration. Stomatal density in the leaves of seedlings increased whereas the leaf stomatal indices declined under NaCl treatment.

5)   Use of a split root system indicated that under water limitation conditions, through PEG, root halves acted independently. Leaf stomatal patterns resembled those of the control tissues, rather the PEG-treated tissues when water was available to one root half. The results, however, did not rule out conclusively that a non-hydraulic signal was involved in stomatal development.

6)   PEG treatments resulted in an reduction of coleoptile growth as well as stomatal density but no reduction in guard mother cell index. Stomatal index showed greatest reduction when both caryopses and root were removed from the coleoptile. ABA treatment showed a reduction in both stomatal index and guard mother cell index in wheat coleoptiles.

7)   Exposure of coleoptiles to light resulted in significant reduction in the length. There was a slight decrease in stomatal density per coleoptile at the same time.

8)   The roots of wheat seedlings produced yellow a pigmentation in response to PEG-induced stress. This pigmentation was due to the production of a single compound, and not to a stimulation of general carotenoid synthesis. Tentative chemical identifications by TLC, absorption spectra and Mass Spectrometry indicated that the pigment could have been one of two compounds:

1,2 bis-3,5-ditert butyl-4- hydroxyphenyl or

3,3-tetra tert butyl stilbene 4,4' quinone.

9)   The pigmentation response indicates that care should be taken when any experiment using PEG to limit water availability since the compound may have had a metabolic effect other than a limitation of water uptake.

The mechanisms that are important in determining plant tolerance to water deficit are not clear The complexity of response suggests that multiple changes in gene expression are drought-induced. There appears to be a well defined degree of plasticity in wheat plants to water limitation. It is also apparent that ABA is a regulator involved in the control of changes in specific genes expression, e.g., rab genes, which occur in response to water deficit. To identify the genes that allow more drought-resistant genotypes is important for the selection of plants to grow in arid zones and for productivity.

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APPENDIX

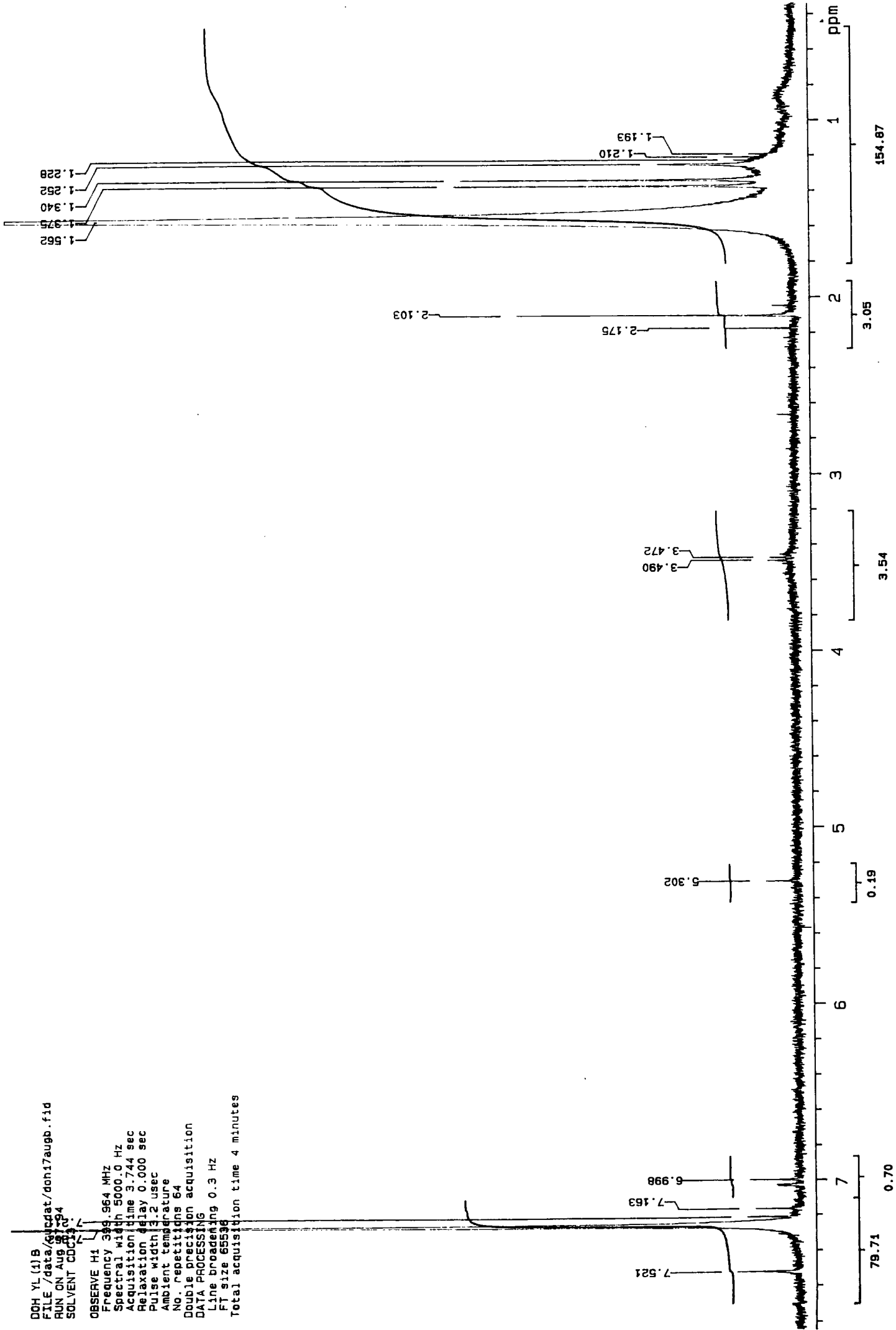Three sample Mass Spectrographs of the pigment produced in wheat roots in response to growth in PEG.

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DATA PROCESSING
Line broadening 0.3 Hz
FT size 65536
Total acquisition time 4 minutes
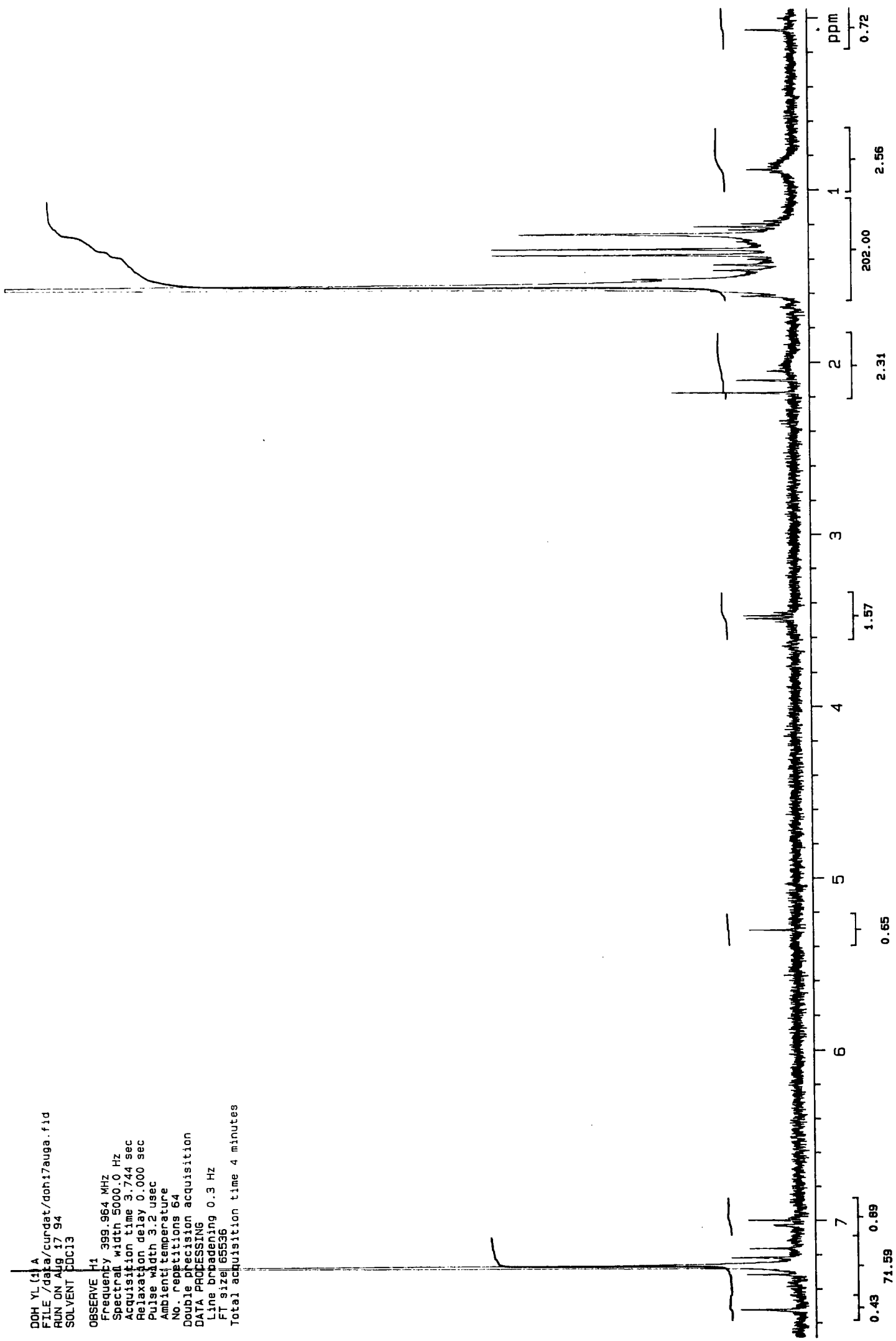
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Double precision acquisition
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1.227
1.209

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