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The Influence of Cycocel on the Responses of Wheat Plants to Water Limitation

by

Ahmed bin Yahya Al-Maskari

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A Thesis submitted to the Department of Biological Science in accordance with the requirements of the University of Durham for the degree of Doctor of Philosophy.

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Thesis

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I DEDICATE THIS THESIS TO MY FAMILY: MY PARENTS; MY BROTHERS AND SISTERS; MY WIFE AND CHILDREN.

ABSTRACT

Considerable research has been concentrated on the foliage application of Cycocel, whereas little attention has focused on its use in seed application. Imbibition of caryopses of wheat (*Triticum aestivum* L.) in (2-chloroethyl)trimethylammonium chloride (Cycocel/CCC) resulted in a reduction in germination rate and a stunting of seedling growth. This was consistent with the appearance of a thicker stem, with shorter leaves than the control. Chlorophyll content per unit weight of leaf and numbers of tillers initiated both increased in comparison to controls. However, Cycocel treatment was accompanied by an enhanced survival capacity of seedlings when they were subjected to water limitation under growth room conditions. Seedlings grown from caryopses imbibed in 0.4 % Cycocel and exposed to a 7-day cycle of watering remained green and turgid beyond the time when the controls had wilted and died. Seedlings, at the fourth leaf stage, showed no wilting 23 days after watering had been withheld totally. Enhanced survival was also noted in seedlings when caryopses had been imbibed in Cycocel for 24 hours followed by a period of dry storage for up to 2 weeks prior to sowing. Enhanced survival was also detected as a carryover into the tillers, and the subsequent GEN2 seedlings, derived from GEN1 plants. Cycocel pre-treatment did not alter Stomatal Index to an extent which could lead to enhanced water use efficiency, nor to the accumulation of proline to bring compatible solutes into equilibrium. However, the pre-treatment with Cycocel was found to enhance growth as a counter to water stress, and maintained fresh and dry weight of shoots when examined under the polyethylene glycol (PEG) induced stress. At the anatomical level, the microscopic investigation of the GEN2 leaf tissues, derived

from GEN1 plant, revealed a fully turgid cell structure, cells did not collapse and tissues were not distorted after the extraction of chlorophyll when compared with the control of the continuous watering as well as the moderate watering regime. Additionally, the cortex of GEN1 plants derived from pre-treated caryopses, showed a bright fluorescing unidentified deposition under Nutrient Solution (NS). This was more much greater when sampled from the NS + PEG treatments.

From the results achieved Cycocel pre-treatment of caryopses appeared to have potential in manipulating wheat plants against water limitation. However, the mechanism (s) by which this can be achieved was not fully resolved in this study. Further investigations are required at the biochemistry and molecular level, particularly in relation to potential carryover effects between generations.

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ABBREVIATIONS

µl	microlitre
µm	micrometer
µmole	micromole
°C	degree Celsius
A	absorbance
ab	abaxial surface
ad	adaxial surface
AO	acridine orange
ATP	adenosine triphosphate
cm	centimetre
Cycocel/CCC	(2-chloroethyl)triethylammonium chloride
E	epidermal cell
EtOH	ethanol
GEN1	first generation
GEN2	second generation
g	gram
GAs	gibberellins
l	litre
M	Molar
M:C:W	methanol:chloroform:water
m^2	square metre
mg	milligram

mg/g	milligram per gram
ml	millilitre
mm	millimetre
mm^2	squared millimetre
mol.	molecular
NADH-GDH	NADH-glutamate dehydrogenase
nm	nanometer
NS + PEG	Hoagland's nutrient solution with polyethylene glycol
NS	Hoagland's nutrient solution
PEG	polyethylene glycol
PGRs	plant growth regulators
ppm	parts per million
R*f*	relative flow
rpm	revolution per minute
s	second
S	Stomata
SD	Stomatal Density
SI	Stomatal Index
TLC	thin layer chromatography
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
wt.	weight

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CHAPTER 1

INTRODUCTION

Within this century the global alert for food necessity has become very prominent. The increase in population and the global environmental changes (in all aspects) have aroused the need to increase agriculture production which, in return, attempts to ensure that food will be provided for millions of people to appease their hunger. Basically, many of the research investigations in plant sciences are a wish to establish a relationship between plant, environment, and food production, therefore many research trends have been adapted and developed. Some of these are, plant engineering, genetic manipulations, tissue culture, and chemical applications. Much of the work is aimed at allowing the establishment of crops on marginal land or in otherwise unfavourable environments.

Since the plant life cycle starts with the germination of a seed and ends up with the production of seeds, we can say that the most conspicuous action of a plant is the growth development. For example, if a seed is viable in the presence of a suitable environment it should develop primordia followed by a canopy and roots, after that the production of flowers, fruits and seeds follows. This build up gives the plant its shape and recognition, therefore, any enhanced internal or external changes caused by the environment to the seeds or the seedling will alter the function of the plants growth and development. The plant responses to any cause is an individual characteristic which depends upon the physiological abilities of plants, and the environmental factors within the plant media.

Wheat (*Triticum L.*), has been one of the humankind food sources since the establishment of early agriculture, it is extensively grown and produced in great quantities (Evans *et al.*, 1978). It is regarded as one of the important crops in international trade, global production amounts to more than 538 million tons per year (Food and Agriculture Organisation, 1990). Wheat is also considered as one of the most widely cultivated cereal crops, because of the plant's wide range of physiological changes and ecological acclimatisations. Evans *et al.*, (1978) stated that if wheat is grown at low latitudes (at about 30°) the crop's vegetative development will take place in the cool short winter days (10 to11 hours daylength), with a relatively low light intensity, while the grain filling takes place at day lengths of 13 to 14 hours. Moreover, if a spring- sown crop is grown at latitudes around 60° or more the life cycle of the crop will go under longer days, with a rise in temperature, radiation, and frequently water stress, whilst grain filling could be completed during midsummer. On the other hand, rapidly increasing temperature with a daily average of 30 °C or more, and increasing water stress had been reported to often terminate grain filling (Evans *et al.*, 1978).

Triticum aestivum L., a species known as bread wheat, is an annual crop that is widely spread and relatively well-adapted to a wide range of climates and soil types. According to Purseglove, (1992) the plant is grown at higher altitudes of the tropics as well as the lowlands and also grown as a winter annual in milder climates. Even though wheat is adapted to a range of soil types, fertile soil with reasonable drainage and good water holding capacity have been suggested to be the preferable growing medium.

Breeding of the crop has further increased the yield potential, and expanded the range of physiological variation and ecological adaptation of the wheat crop. To

produce adapted high yielding cultivars, Purseglove, (1992) indicated the wide range of wheat cultivars that have been bred in temperate countries, these include winter and spring wheat. Likewise, breeding has been carried out in subtropical and at higher altitudes of the tropics to produce: a) locally adapted cultivars, and b) a wheat rust resistance, such as the feature seen in Kenya's wheat production. Furthermore, efforts have been made to produce cultivars adapted to low rainfall season areas.

However, the most prominent feature of all breeding programs is the production of the dwarf cultivars at the wheat improvement centre in Mexico. This crossing of dwarfing genes of the Japanese 'Norin cultivar' endowed the ability to support a heavier yield without collapse of the plant (Purseglove,1992).

The principle use of wheat is for flour, where the grain is ground into flour and used for bread. Grain is also used as a source for cakes, biscuits, pasta etc. Wheat is also used as an alternative feed for animals, where straw of the wheat provides a valuable fodder for livestock, and can be used as pasture. It can be grazed only in emergency (as winter feed or at a crop failure) during the vegetative phase. It is normally grazed before the stem elongation (Loomis and Connor, 1992). Establishment of the crop, therefore, is of paramount importance and the early growth of seedlings is one which has a potential for manipulation.

In many cases, observed changes in the physiology and development of plants results from an environmental stress. The physical environment in which a crop is grown is recognised as the main determinant to what crop will grow and has a large effect on the type and rate of growth. For a plant physiological process to take place minimum requirements of solar energy, water, and nutrients are needed.

In general, we can define plant stress as a disadvantageous state caused by external environmental factors. Whatever the cause is, for the plants to keep pace with the environmental changes it has been noted that some will acclimate to the changes up to a point. The lack of control and the variable nature of the environmental physical conditions including temperature, water, light intensity, gases, and acidity impose a limitation on seeds and seedlings. These effects can include an impairment of the initiation of seed germination and the breaking of the dormancy period, and restraint or protraction of the plants normal function.

The effect of environmental factors on the plant parts are very well documented, however, in this section we are highlighting only some aspects of the temperature and water deficit due to the importance of these two to the subject concerned. Thus it cannot be inferred that these components are independent factors, they interact with the rest of the environmental factors to either limit or promote plant growth and development.

Every plant has an ideal temperature for growth (McDaniel, 1982). Most plant seeds germinate readily at a temperature of 6 to 10 °C but the range varies considerably, for example; wheat seed germination will occur between 4 and 37 °C, and the optimum percentage can be obtained at a temperature of 20 to 25 °C (Harper, 1983). It has also been noted by Aldasoro *et al.*, (1981) that a temperature of 30 °C will induce a delay in the germination of chick-pea seeds. Similar work by Revilla *et al.*, (1988) showed that the optimal germination of a chick-pea seed is 25 °C, and at 30 °C the seed germination will be delayed for 24 hours.

Temperature has been widely reported to affect the growth and development of most plants parts. Some of there effects are seen on leaf growth and expansion, rate of development of root system, photosynthesis, and as a major factor in opening and

closing of stomata. It has been reported by Meyer *et al.*, (1960) that stomatal opening in cotton and tobacco increases with a rise in temperature upto 25-30 °C, while it decreases at still higher temperature. Harper, (1983) indicated that photosynthesis and development in the maize leaves were slow at 10 °C and fastest at 30-33 °C. So temperature is a factor that accounts for water shortage occurrence in plants whenever water loss through transpiration exceeds supplies.

Water scarcity is a major component of the environmental strain in many areas of wheat crop production. Since water is essential for growth and development of the plant, lack of water during the activity phase may cause many changes in the metabolism and result in unbalanced growth. As a consequence, cell growth, cell wall expansion, and protein synthesis are adversely affected (Harper, 1983). Stomatal closure occurs in the absence of an adequate water supply, leading to the reduction in the CO_2 exchange and hence photosynthesis. Water stress can result in the reduction of the crop yield, and if water shortage persists, permanent wilting and death of plants may result. On the other hand, prolonged exposure to excessive water can also damage the crop. High humidity in the atmosphere can also increase the risk of plant infection by some plant disease organism (Harper, 1983).

Lodging of wheat (blowing over by the wind), is one of many important characteristics that was considered as a major constraint of the production system (Wiersma *et al.*, 1986). Harper, (1983) indicated that lodging of cereals and other crops is induced by stem diseases and adverse weather conditions, however, in the absence of stem disease, stem characteristics are said to be the determinants of lodging incidence.

Hay and Walker, (1989) stated that the development of larger leaves and taller stems in a heavily fertilised crop make it more prone to damage by lodging and various types of pathogenic attack leading to the possibility of yield loss. At high plant population densities, thinner and weaker stems occurring in stands provides a more favourable environment for lodging damage, and this is associated with adverse weather conditions of wind and heavy rain (Harper, 1983).

The lodging of crop plants, the associated grain loss and the increase incidence of diseases has set the aim of breeders to manipulate the genetic variability in a crop, and to breed cultivars to improve plant characteristics such as the production of shorter and stronger stems to avoid environmental problems. However, these manipulations and breedings are very costly in terms of time and resources (Hay and Walker, 1989). Even though the aim of breeders over the last 30 years has been to develop the lodging-tolerant cultivars with shorter and stronger stem (Hay and Walker, 1989), the production of short stiff-strawed cultivars has not abolished totally the problem of lodging (Pinthus, 1973). This is due to the trends facing shorter cultivars of the strong affinity between plant height, possible grain yield, and total biomass (Austin *et al.*, 1980). The interest in alternative methods of the control of plant lodging has been significant, leading to the development of commercial plant growth regulators, which act as stunting and anti-lodging agents. Cox and Otis, (1989) stated that lodging can still be a problem in the production of wheat, and the need for the use of plant growth regulators to control lodging does exist. In this context the potential use of natural plant growth regulators (PGRs) and related compounds has been the subject of some investigation.

PGRs are usually defined as organic compounds which affect the physiological processes of the plants and their action is concentration dependant. PGRs can be

classified according to their source i.e. natural or synthetic, and to their function either promoter or retardant. Experimental work with PGRs has indicated the ability of the compounds to influence each of the individual parameters which contribute to water uptake into the cells, plus those which are involved with the capacity of the cell wall to extend (Jones and MacMillan, 1984).

The major component of plant stem elongation has been ascribed to the internal response to the increased level of endogenous gibberellins (Hay *et al.*, 1989). The gibberellins (GAs), are known for their widespread occurrence in plants, their ability to induce spectacular growth responses, they are accepted as hormones in higher plants (Jones and MacMillan, 1984). Many researchers have highlighted the role of GAs in the promotion of growth of the plants by affecting either cell expansion or cell division, or both. Applied GAs cause an increase in the diameter of many conifer species stem, promote shoot elongation and induced flowering (Jones and MacMillan, 1984). Loy, (1977) also noticed that in the sub-apical region of both rosette and caulescent plants that GA_3 increases the size of the meristematic region and the proportion of cells which are undergoing division. They are also reported to accelerate leaf expansion especially in young plants, and if applied to genetically dwarf plants, promote the growth of stems to a normal height (Harper, 1983).

These chemicals also have very specific effects on plants eg. they are also ascribed to activate the production of α -amylase enzymes in the aleurone layer of the seed. α -amylase is a hydrolytic enzyme, which is involved in the conversion of starch in the endosperm to simpler sugars, and the production of other solutes, which are then transported to the growing embryo (Chrispeels and Varner, 1967).

Many research interests have been directed to the production of substances that can counteract gibberellin biosynthesis or their action as a means of manipulating plant

growth. Consequently, out of the very many chemical compounds which have been screened for activity, (2-chloroethyl)trimethyl ammonium chloride (Cycocel/CCC) is one which has been developed for practical use in agriculture. It is mainly used as a plant growth retardant, and is manufactured under a variety of trade names (Budavari *et al.*, 1989).

The effect of applied Cycocel on crop height, lodging, drought tolerance, and yield have been investigated by many researchers (Tolbert, 1960; Halevy and Kessler, 1963; Humphries, 1963; Amoabin *et al.*, 1986; and Bishnoi *et al.*, 1994; Olumekun, 1996). Halevy and Kessler, (1963) stated that plants treated with Cycocel were less susceptible to water stress than controls (untreated). They also found that high temperature followed by low light intensity exposure of bean plants caused wilting in control plants, while the treated plants remained turgid. Other work by Bishnoi *et al.*, (1994) pointed out that high temperature following low light intensity resulted also in wilting of control plants of Bajra cultivar, while Cycocel-treated plants remained turgid. This work indicated the capability of Cycocel to improve water status and yields of Bajra. Moreover, those plants which were treated with the retarding substance, depending on the concentration, either displayed a delay in the growth or showed an increase in the development of meristematic activity and roots.

Tolbert, (1960) indicated that the most distinct feature of growth after Cycocel treatment was the production of a shorter plant, with a thicker stem and darker green leaves than the control. Similar effects of Cycocel on a variety of crops were reported by Cathey and Stuart, (1961). Olumekun, (1996) stated that Cycocel mainly influenced the size rather than the number of plant parts, and reduced relative growth rate of dry matter accumulation in winter wheat. These results are in agreement with the finding of Bruinsma, (1982) that Cycocel acts to slow down the rate of growth.

These alterations in growth did not, however, affect total dry matter accumulation (Tolbert, 1960; Tabora and Hampton, 1992).

Olumekun, (1996) reported that a foliar application of Cycocel at the beginning of stem elongation reduced the culm length by 19.8% at terminal harvest. This reduction was associated with reduction of the first three internodes by 43, 37, and 15 percent respectively, this shows that the influence of Cycocel on stem growth decreases with age. These observations support the suggestion of Tolbert, (1960) that Cycocel is not broken down rapidly within the plant but is translocated into the new leaves and persists.

While the majority of the studies emphasise the ability of Cycocel to retard plant growth (Cathey, 1964; Sachs and Kofranek, 1963; Weidner, 1987) only a few experimental works have shown that small doses of Cycocel solution can significantly stimulate or increase growth (Reid and Crozier, 1970; Ojeda and Trione, 1994). Guttridge, (1966) stated that a small dosage of Cycocel promoted the growth of strawberry plants, and similar work on pea seedlings by Adedipe *et al.*, (1968) indicated the effectiveness of Cycocel to promote growth at low concentrations. From two-year field experiment Tabora and Hampton, (1992) noted that Cycocel treatment to *Lotus uliginosus* Schk cv. Grassland Maku did not retard the shoot growth, or affect the total dry matter, nevertheless treatment increased pods per umbel and seeds per pod, leading to the increase in seed yields. This inability of Cycocel to retard internode length was also detected by Niemelainen, (1987) in red clover legumes, though treatment increased seed yield in the first year of two successive cropping years.

The apparent conflict in results in the effects of the growth substance indicates that Cycocel may be species-specific and may be even cultivar-specific (Tabora and

Hampton, 1992). This can also be attributed to the selectivity and variability of various plant organs, concentration and methods of Cycocel application, duration of treatment, and the concomitant environmental growth conditions (Tolbert, 1960; Halevy *et al.*, 1966; Bode and Wild, 1984).

The effect of Cycocel is beyond controversy on the stunting of growth in some plants at specific concentration, however, related developmental changes may also be induced eg. in the proliferation of tillers. A similar related phenomenon, is seen in the grazing and cutting management of pasture crops which shows a profound influence on the stimulation of tillering of grasses. Tolbert, (1960) reported that Cycocel treatment of young wheat plants (in greenhouse conditions) resulted in an early tiller initiation shortly after treatment. The tillers grew almost as rapidly as the main culm, but tiller formation at the time of heading was not the same as for control plants which developed tillers near the time of heading. Ma and Smith, (1992) stated that an early application of Cycocel tends to increase tiller numbers by increasing tiller production in spring barley, whereas late treatment increased the number of tillers bearing spikes. This may be attributed to the availability of more assimilates for the tiller bud initiation and subsequently growth occurred during main shoot suppression. This effect may be related to the observation of Naylor *et al.*, (1986) who suggested that Cycocel application to barley crops reduced the sink capacity of the main stem thereby freeing assimilates to contribute to growth of other plant parts. On the other hand, Kettlewell *et al.*, (1983) reported that Cycocel had no effect upon the number of tillers per plant of winter wheat, but it increased the number of ears per square meter.

Treatment with Cycocel has also been reported to have a carry-over effect on the growth of tillers. Pinthus, (1968) stated that Cycocel treatment of wheat at the time of spikelet differentiation onward caused a considerable and significant decrease

in the length of tillers. Bishnoi *et al.*, (1994) stated that treated seeds of Bajra which were grown under rain-fed conditions increased the number of ears bearing tillers per plant but decreased the ear length. It has also been reported by Plaut and Halevy, (1966) that Cycocel has promoted the recovery of wheat tillers after a period of water stress.

The mode of action of Cycocel in its inhibition of gibberellins is not totally resolved. It is generally accepted that the gibberellin biosynthetic pattern is blocked at the production of the precursor of (ent)-Kaurene (Robinson and West, 1970). This observation was confirmative of an earlier work by Baldev *et al.*, (1965); and Harada and Lang (1965). A study by Reid and Crozier, (1970) indicated that application of low concentrations of Cycocel to pea plants increased the endogenous levels of gibberellins without any noticed changes on the growth development, conversely a high concentration of the same hormone did not alter the GAs level in the plants. Similar work by Prasad and Prasad, (1994) showed the ability of Cycocel to adversely affect the growth and yield of cotton. Such growth pattern results have been emphasised by many investigators to be opposite to those of gibberellins (Tolbert, 1960; Baldev *et al.*, 1965; Harada and Lang, 1965).

Actions other than on GA synthesis have also been proposed for Cycocel. Halevy *et al.*, (1966) reported in their experiments on detached plant organs that the growth retardant may be non-specific and probably has an indirect effect on the respiration. It was also indicated that the effects on respiration were species-specific and were dependent on the external conditions. For example, CCC was found to be effective in depressing respiration at 20° and 15° for lettuce and bean leaves, and some flower cultivars while not for broccoli, mushrooms and other flowers cultivars.

Dry matter accumulation underpins all aspects of plant growth and this relates to photosynthetic capacity. All higher plants are distinctive by the fact that their leaves contain pigments, one of the most frequent and obvious is chlorophyll. Chlorophyll, is a light-absorbing pigment, located within the chloroplasts, it gives the green colour to plants and algae, and is known to convert light energy into chemical energy in the process of photosynthesis (Wallace *et al.*, 1996; Campbell, 1996). It has been reported by Rudiger and Schoch, (1988) that the ability of bacteria and plants to produce chlorophyll seems to be restricted to the photosynthetic organisms, it is also noted by Maksymowych, (1973) that the photosynthetic rate is proportional to the chlorophyll concentration within the cell. The overall process of photosynthetic reaction determined is by three process; diffusion of CO_2, light interception, and the reductions of high energy compounds which are used in the production of carbohydrates.

The overall dry matter of the plant has been reported by Milthrope and Moorby, (1979) to consist of 85-90 % carbonaceous material derived from photosynthesis. In another words, Rudiger and Schoch, (1988) stated that "photosynthesis in its present form cannot be imagined without chlorophylls". In addition Neale and Melis, (1989) reported that a decline in photosynthesis rate is often associated with a decline in growth as it has been measured by the CO_2 assimilation.

Since chlorophylls are specialised light absorbing pigments in the photosynthetic process, it needs to be noted that there are a number of different types of chlorophyll which occur in the plant domain. They are summarised by Meyer et al., (1960) and Allen, (1966) as chlorophyll a, b, c, d, bacteriochlorophyll and bacterioviridin. These pigments absorb only visible light ranging from 380_{nm} to 750_{nm} in wavelength, thus different pigments absorb light at different wavelengths. All of these compounds contain magnesium, and are very similar in chemical structure

(Meyer *et al.*, 1960). Chlorophyll a and b are the normal chlorophylls of higher plants, while chlorophyll a is the most dominant in all oxygenic photosynthetic organisms (Rudiger and Schoch, 1988). Burger-Wiersma *et al.*, (1986) reported that chlorophyll b is a constituent of the light harvesting mechanism in higher plants, algae, Euglenaceae and prochlorophyta, and is generally present at approximately one third the concentration of that of chlorophyll a. Any environmental factor which will influence the level and availability of chlorophyll will, by definition, influence photosynthesis and ultimately yield. The availability of chlorophyll in plants is subject to many factors, some of which as summarised by Meyer *et al.*, (1960) are genetic factors, light, oxygen, mineral elements, and water

In addition to the direct effects on the growth process in plants Cycocel has also been shown to influence the basic photosynthetic capacity of plants through the availability of chlorophyll. Treatment with Cycocel significantly increased the chlorophyll content of cotton leaves (Bhatt and Ramanujam, 1970), per leaf and per unit area in tobacco plant when grown in a culture solution (Humphries, 1963), and the content per unit leaf area in the guayule (Ojeda and Trione, 1994). However, in tobacco the net assimilation rate was reported to be reduced, which could be attributed to the inhibition of stem growth (Humphries, 1963).

Likewise Bode and Wild, (1984) observed a decrease in the photosynthesis rate of wheat leaves following Cycocel treatment, however, the pigment concentrations were not influenced by the treatment i.e. chlorophyll a, and b were present in almost similar amounts as in the control plants during the development of the third leaves. Cycocel treatment also resulted in a rise in the diffusive resistance of the leaves but very little difference was found in the concentration of the internal CO_2 compared with the control.

In contrast, Kuznetsov *et al.*, (1992) reported that Cycocel increased the accumulation of chlorophyll a and chlorophyll b content in the primary leaf of wheat from 45 to 77.6 and from 11.0 to 19.6 mg per leaf respectively. This increase was ascribed to a response of Cycocel in the presence of short-term red light which could activated the synthesis of chlorophyll, the formation of RC PS II and the electron transport reaction. In addition, seeds which were pre-treated with Cycocel also showed the absence of photoreversibility of red light effects by far red light, related to a phytochrome response (Kuznetsov *et al.*, 1992).

One of the major environmental features which influences growth and development, and productivity, of plants is the availability of water. Among the plant adaptations to water stress, leaf modification is an important component in the long term changes to improve plant tolerance to drought (Parkhurst and Louks, 1972; and Pugnaire *et al.*, 1994). Rapid leaf expansion may expose water availability to excessive loss through transpiration. Prakash *et al.*, (1992) stated that 97 % out of the total water absorbed by plants will be lost through transpiration. A report by Bode and Wild, (1984) indicated that in wheat plants final leaf size was reduced by 40 % under the influence of Cycocel, and this reduction could be related to the leaf manipulated cell division frequency. It was also noted by Prakash *et al.*, (1992) that application of Cycocel to moisture stressed brinjal plant leaves decreased leaf temperature, and transpiration, but increased diffusive resistance up to the level of 50 % compared with the unstressed plants.

Research investigation on developing artificial techniques to either alleviate drought stress effects or induce drought tolerance in plants have been undertaken

(Balki and Padole, 1982), in which the application of Cycocel is an example. Some studies have indicated that Cycocel is able to restrict water loss between the canopies and the atmosphere by means of increasing the plants drought tolerance (Halevy and Kessler, 1963), or by improving the plant water status and yield (Bishnoi *et al.*, 1994). Generally, the application of Cycocel has induced a range of responses to which a plant is subjected, depending on the concentration and the methodology in use.

It is well known, by now, that water stress primarily causes stomatal closure in plants, to what extent depends on the water availability either permanent or temporary. As a response to the drought stress, plants either adapt or acclimate to their environments, accordingly several adjustments in the plant structures and processes are recognised as taking place, some of which, beside the hormone and enzymes production and activity, are stomatal sensitivity and osmotic adjustment, smaller cell volume, reduction in the leaf area, increased leaf thickness, and changes to the root-shoot ratio (Pugnaire *et al.*, 1994). For example, Simmelsgaard, (1976); Jones and Turner, (1978); Zagdanska, (1984 a & b) stated that the stomata of a drought-hardened leaves of maize, sorghum, cotton, and wheat remained open at an induced low water potential, resulting in a relatively high photosynthetic rate which could be related to the lowering of stomatal sensitivity under stress conditions. On the contrary, Bode and Wild, (1984) ascribed stomatal closure to a possible adaptation mechanism of plants which are exposed to water deficit.

The environmental factors which effect the stomatal movement are discussed in detail by (Weyers and Meidner, 1990). These environmental factors are: direct and indirect irradiance, temperature, carbon dioxide concentration, air pollution, wind, nutrition and diseases, and the water supply.

Stomatal closure is a common defence mechanism against drought stress which allows plants to exploit a more stressful environment. However, to what extent Cycocel is involved in the stomatal closure is unclear. In this context Stalfelt, (1955) stated that as mild water stress develops, there is a remarkable loss in solutes from guard cells resulting in stomatal closure. On the other hand, Govil, (1985) described the Cycocel effect as; (1) acting directly on guard cell membrane, or (2) acting through the synthesis of proline of glycine-betaine that could affect the membrane leading to reduced potassium in guard cells and thus stomatal closure.

Water content of a plant is largely dependent on stomatal index (Gupta, 1992), and the stomata are regarded as portals through which the water vapour diffuses out in a large quantity through the same route that CO_2 and other gases diffuse in (Hay and Walker, 1989; Sen and Mohammed, 1994). A study by Gupta, (1992) indicated that Cycocel treatment of the seed of *Vicia faba L.* increased the epidermal cell and stomatal size at higher concentration, while it decreased their frequency, it was also shown that the stomatal index could be reduced with a Cycocel application concentration of between 0.005 to 0.05 %. Thus a manipulation of stomatal numbers per leaf by chemical treatment could have a significant influence on water loss.

Further evidence of Cycocel application in the manipulation of water relation in growing plants has been found. Application of Cycocel to a pre-sown seed of pearl millet resulted in the increase in the values of relative water content, with a significantly increase in the leaf water potential Bishnoi *et al.*, (1994). These growth responses of plants have been attributed by Singh *et al.*, (1973); and Bishnoi *et al.*, (1994) to a number of possible causes. A change in plant water uptake, which is supported by Primost, (1968) who stated that growth retardants allow plants to absorb more water. The alteration of the rate of water diffusion, which agrees with the

observation of Bode and Wild, (1984) that Cycocel treatment increased the diffusive resistance of young wheat plants. This was further supported by the experimental work of Prakash *et al.*, (1992) with brinjal plants leaves. Cycocel could have caused a change in the balance of water within the plant cells, this was supported by Amoabin *et al.*, (1986) who showed that treating tomato plants with Cycocel resulted in their being able to sustain more water potential under progressively more severe water stress. However, Cycocel treatment did not affect the water potential of well-watered plants.

In contrast to the effects caused by water stress on the vegetative growing plant parts, water stress is also involved in the inhibition of protein synthesis and the modification of amino acid metabolism leading to the accumulation of amino acid proline (Barnett and Naylor, 1966; Singh *et al.*, 1973b). Proline accumulates in many plant leaves under different stress conditions (Miflin, 1980). This accumulation has been attributed to protein breakdown (Levitt, 1980) and also from glutamate synthesis as glutamic acid known as a precursor for proline biosynthesis (Agarwal *et al.*, 1994). A key enzyme in proline synthesis, NADH-glutamate dehydrogenase (NADH-GDH) has been reported to exhibit higher dehydration tolerance during water stress (Miranda-Ham *et al.*, 1985). On the other hand, NADH-GDH activity in pigeon pea has been stated to be depressed in roots and nodules under water limitation (Sheoran *et al.*, 1981). However, the activity of NADH-GDH has been reported by Srivastava and Singh, (1987) to depend on species, plant organ, growth stage and water limitation.

It has also been stated by Singh *et al.*, (1973) that proline, during water stress, is capable of accumulating within a few hours, then it rapidly declines when water stress is ceased. Though proline accumulation is quick and extensive in the leaves, its

accumulation could occur in other organs of the plants, and the rate and quantity of accumulation varies between different genotypes which are held under similar water stress conditions (Sing *et al.*, 1973). Proline production and/or its accumulation is a pronounced water stress response to provide one class of so-called compatible solutes (osmolytes), which are present in all organisms and are osmotically active.

Since proline is considered to be one of the compatible solutes that accumulates in the cytoplasm, it is believed to maintain the osmotic potential equilibrium in plant cells (Kauss, 1977; Taiz and Zeiger, 1991; Hu *et al.*, 1992; Delauney and Verma, 1993), and is involved in the cytoplasmic adjustment in low water potential (Agarwal *et al.*, 1994). The indication of proline accumulation may serve as a sign of an initial protection effect for stress tolerance, or of damage at higher levels of accumulation (Levitt, 1980; Miflin, 1980; Salisbury and Ross, 1985). Many other investigators have reported that not all plants accumulate proline at the same degree of stress. For example, Lawlor and Fock, (1977b) stated that sunflower accumulates proline under severe stress only. This was also reported to hold for sorghum and soybean (Waldren, *et al.*, 1974).

The presence of proline during water deficit is not only restricted to that of it being an osmolyte, it has the potential to serve in organic nitrogen storage and, in the mitochondria, it is directly coupled to the respiratory electron transport system and the production of ATP (Elthon and Stewart, 1981). It has been suggested that it assists in improving the energy status of a plant during recovery from stress (Lawlor, 1995).

Therefore, prolonged inadequate water supply affects virtually all metabolic activities in addition to the severe reductions in plant productivity (Bohnert *et al.*, 1995). Thus an alternative method by which growth retardants could enhance a plant's survival from water stress, is the modification of metabolic response of plants, in

particular the accumulation of specific metabolites (Singh *et al.*, 1973). A study by Agarwal *et al.*, (1994) indicated that Cycocel treatment of *Lablab purpureus L.* under PEG-induced water stress significantly increased the level of proline in leaves, and slightly increased the activity of NADH-glutamate dehydrogenase in roots and cotyledons. Similar work on suspension-cultured cells of tomato indicated that there was an increase in free proline under PEG treatment conditions (Handa *et al.*, 1983). However, Singh *et al.*, (1973) indicated in their work with wheat plant grown in PEG solutions that CCC treatment had no direct influence on the level of free proline accumulation, but an indirect effect through the increase of leaf tissue capacity to accumulate higher concentration of proline during water stress. It is also stated by Singh *et al.*, (1973) that under no water stress CCC did not affect the free proline concentration in the plant leaves.

Many investigatory works have demonstrated that proline could actually be beneficial to a plant during water stress, however, the manner, and at which point, Cycocel may influence the response of plants to water deficit, is still a subject of controversy.

As in the case with proline, water stress is also capable of leading to the induction and accumulation of proteins. It has been stated by Dubey, (1994) that water stress is capable of changing the gene expression in plants, which in turn leads to an inhibition of the synthesis of protein in general end to the enhanced synthesis of stress-specific proteins. In general, plants which are subjected to water stress exhibit a decrease and an increase in the cellular proteins according to the severity of the stress (Dubey, 1994). In 1964 Todd and Yoo reported that there was a decrease in the protein level of detached wheat leaves as well as the activity of several enzymes in response to limited water availability. Similar work on sugar beet leaves showed a

gradual decline in the soluble and total protein within days after water cessation (Shah and Loomis, 1965). Additionally, water withholding from Bermuda grass resulted in a general decrease in the soluble protein as well as a decrease in the insoluble protein (Barnett and Naylor, 1966).

Of interest, Halevy, (1967) indicated that Cycocel delayed the breakdown of nucleic acids, protein, and of leaf senescence. Such effect have lead to the increase of protein content (Kharanyan, 1972). Bode and Wild, (1984) have stated that even though CCC retarded the vegetative growth, it did not inhibit the metabolism of wheat third leaves. Thus, in contrast to the control, CCC treatment in young wheat plants significantly increased the level of soluble protein and soluble reducing sugars. A similar stimulatory action was also seen on the synthesis of RubPc-ase protein and on the *in- vitro* activity of this enzyme. The protein increase was attributed to the Cycocel enhancement of the *in vitro* RubP-Carboxylase protein activity, which can account for 50 % of the soluble protein content in a photosynthetic plant cell (Bode and Wild, 1984). The CCC stimulation of the *in vitro* RubP-Carboxylase activity has also been reported by Abdelhadi *et al*., (1980); Marcelle *et al*., (1973); and Wellburn *et al*., (1973).

Although Cycocel was initially employed as a crop lodging controller, it is now considered as a source of multiple function, for example as a tiller enhancer and a yield increaser. In addition, there is evidence to indicate that it has effects on metabolism, which related to growth, are more applicable to the survival of the plants itself. In this context, it is possible that Cycocel can moderate the effects of environmental stress in plants. Given the importance of the manipulative effects of the compound are justified.

In general, much of the present research is directed at the behavioural responses of plant tissues at different stages of Cycocel application rather than studying the structural plant parts. The use of the growth retardant "Cycocel" has been said to alleviate stress and avoid injury or death of plants, however, to what extent the retardant is involved into the morphological structure of the leaves, and root system of a plants needs to be exploited.

1.1 AIMS:

Cycocel appears to have a number of diverse effects on higher plants, one of which appears to be the potential to protect against limited water availability. In this context, it is unclear as to how the growth regulator is able to act in this role. Preliminary investigations of the action of Cycocel indicated that it could manipulate both the structure and metabolism of plants in order to be effective (Alaib, 1985). The aim of this work was to investigate further the action of Cycocel as a protector against water stress using wheat seedlings as a model system.

The protocol of investigation was to characterise first all the extent to which plants could be protected, particularly through pre-treatment of seeds. Following this it was intended that the effect of Cycocel should be examined on the morphology, anatomy and metabolite accumulation of wheat emphasising there features which would maintain water availability in plants subjected to water stress conditions.

CHAPTER 2

MATERIAL AND METHODS

2.1 PLANT MATERIAL AND GROWTH CONDITIONS:

2.1.1 Plant Material

Seedlings of *Triticum aestivum* L. Mercia, were used as experimental plant material. Caryopses were obtained commercially.

2.1.2 Growth Conditions

Seedlings were grown under a controlled growth room environment (25/16 °C day/night temperature and exposed to 16 hours photoperiod at a light intensity of 140 $\mu mole/s/m^2$).

Wheat caryopses were soaked overnight at 25 °C; either in water or treatment solution. The pre-soaked caryopses were sown in 90-mm Petri-dishes on filter paper and 10 ml of aqueous solution. The Petri-dishes were then placed in a 100 % relative humidity room for 7 days in darkness at 25 °C to allow germination. Seedlings were then transferred to either soil, or hydroponic media as required.

2.1.2.1 Soil

Levington's 3M compost and vermiculite were obtained locally. Three of the pre-germinated seedlings were sown in individual 10.8 cm. plastic pots containing ¾

Levington's compost mixed with ¼ vermiculite. The latter was used to improve the soil aeration and drainage; which in return gave a better root growth and penetration.

The soil experiment consisted of two watering regimes as follows: (1) water-stressed (water withholding) where wheat plants was irrigated every 7 days until the full expansion of the fourth leaf. (2) continuous watering (control) where plants were irrigated every 2 days throughout the experiment.

2.1.2.2 Hydroponic culture

This technique involved two applications: 1) non-stressed, where nutrient culture solution was used as a substitute for soil, and 2) stressed, involving the use of polyethylene glycol (PEG) as an induced osmotic potential medium dissolved in the Nutrient Solution.

Five of the pre-germinated seedlings were planted through holes in the plastic lid of honey jars and were grown under the controlled growth room environmental conditions (above). The plant element requirements were provided by Hoagland's solution (Table 1). All chemicals were supplied by Sigma chemical company, unless otherwise specified.

Hoagland's solution was contained in 500 ml capacity honey jars; the glass was covered with aluminium foil to prevent any algal growth. The plastic cover lid of each jar was drilled with 8 holes. Seedlings were inserted through the holes and held in place with cotton pads. Care was taken to avoid any unnecessary pressure that could be caused to the stem.

Table 1 Composition of Hoagland solution, used for wheat seedlings grown in hydroponic culture.

Hoagland's Solution Salt	Molarity (M)	mg/l (ppm)
KNO_3	0.006	
$Ca(NO_3)_2$	0.004	
$NH_4H_2PO_4$	0.001	
$MgSO_4.7H_2O$	0.002	
Mixture of 0.5 % $FeSO_4$ and 0.4 % tartaric acid: 0.6 ml/l added 3 times/week.		
$MnCl_2.4H_2O$		1.8
H_3BO_3		2.86
$ZnSO_4.7H_2O$		0.22
$CuSO_4.5H_2O$		0.08
$H_2MoO4.H_2O$		0.09

2.2 PLANT TREATMENTS:

2.2.1 Cycocel Pre-Treatment

Wheat caryopses were imbibed and germinated in 0.004, 0.04, and 0.4 % of the following.

2.2.1.1 Commercial Cyanamid

An aqueous solution containing 40 % w/v Cycocel was used as a stock which was diluted with distilled water to the desired volume to give the percentage strength required. Supplied by Cyanamid of Britain, Agriculture Division, Gosport, Hampshire.

2.2.1.2 Chlorocholine Chloride Salt

Two grams of chlorocholine chloride ([2-chloroethyl] trimethyl ammonium chloride) were dissolved in distilled water and brought up to 100 ml volume to give 2

% concentration. The percentage strengths required were then prepared from the standard solution by dilution. Supplied as a solid by Sigma Chemical company.

Stock solutions prepared from both sources were refrigerated at 4 °C until required.

2.2.2 Stress Treatment

In order to do this, two applications were utilised.

2.2.2.1 Pot Experiments

Routine irrigation of the pots was carried out every 2, 4 or 7 days bringing the soil to field capacity. Plants were watered under these regimes until the full expansion of the fourth leaf, at which time water supply was ceased. After that, if necessary, plants were left to grow until the complete wilting of the control (untreated) plants. Quantitative analyses of shoots, roots, and soil were implemented as required. For each treatment three plants were grown in each of three replicate pots.

2.2.2.2 Polyethylene Glycol (PEG) Experiments

The pre-germinated seedlings were grown hydroponically in an aqueous solution of PEG. A fresh solution of PEG (mol. wt. 8,000) was made for each experiment that was implemented. The osmotic solutions were prepared according to Resnick (1970); 125 g of the salt was dissolved in Hoagland solution. The solution was then brought up to a litre volume to give a water potential of -0.5 MPa, this concentration allowed stunted growth of the plants, higher concentrations inhibited

growth completely. The experiment was carried out under the controlled environment condition as per (2.1.2). five seedlings were grown in each of five replicate treatments.

2.2.3 Propagation

In this part of the experiment, wheat seedlings were grown in pots for the production of tillers. Tillers were then separated and propagated in sequence until the third tillers had been produced.

2.2.3.1 Propagation of Tillers

To investigate the effect of treatment on the plant tillers, three of the treated wheat pots, randomly picked, were set aside. Normal irrigation of the pots was carried on every 2 and 7 days, until the production of the first tillers. At this point, and after the full expansion of the first leaf, primary tillers were excised, hand-cut using a razor blade, from the mother plant. After this, three of these tillers per pot were planted in a soil mixture, irrigated with water as above, until the production of the secondary tillers started. These tillers were excised and were grown on as the third generation of tillers.

2.2.3.2 Water Withholding

To determine whether tillers response to water deficit, two pots of each of : primary, secondary, and tertiary tillers were subjected to drought stress (water withholding). This was conducted, until the full expansion of the 4th. leaf, under the growth room environmental conditions. Quantitative analyses of leaves, roots, and soil were then made.

2.2.4 Growth Parameters

2.2.4.1 Leaves

Length of individual leaf blades (leaf 1 to leaf 4) was recorded in millimetres at the time of full expansion of each leaf. Data were collected, analysed, and compared with to the control (untreated) plants.

2.2.5 Plant and Soil Water Content

This experiment included the measurement of water content in shoots, roots, and soil of the plants. Tissue samples of each part were assessed quantitatively as fresh weight and the dry weight derived from this.

2.2.5.1 Water Content in Shoots and Roots

Treated and control plants were exposed to water deficit (water withholding) for a period of time, and subjected to the control growth room environmental conditions. Shoots were excised from the roots, and each sample was weighed and then dried overnight at 70 °C in an air dry oven, then reweighed.

2.2.5.2 Water Content in Soil

Small samples of soil were taken from individual pots, weighed and oven air dried overnight. Accordingly, data of both (plant and soil) were collected, and percent water content and water lost were calculated. There were two pots per sample

randomly selected from each experiment plus control, and each sample included two water regimes.

2.3 ANATOMY

2.3.1 Fluorescence Microscopy Work

A Diaphot fluorescence microscope was used as a tool to study some parts of the plant tissues. Individual specimens of the plant tissues was viewed under blue, blue-violet, green, ultraviolet, and violet excitation filters. This wide range of filters was used to cover the commonly used wavelength, and to check the presence of substances under the appropriate filter system. Afterwards, only selected photographed pictures, and filter blocks were used to record the observations.

2.3.1.1 Leaves

The 4th. leaf only was used for analyses. Each leaf was cut into 3 equal segments; top middle, and base. A thin transverse section was cut from the middle of each segment with a razor blade and was stained with the appropriate stain and was viewed under the illumination of the florescence microscope, to study the cell wall characteristics and cellular integrity and inclusions. Each treatment involved 3 leaf samples, and 2 stains. The samples were viewed and photographed at a magnification of x20 and x40, using a Nikon filter block, and were photographed using 400 Fujicolor film. Each section was stained as follows:

2.3.1.1.1 Acridine Orange

Sectioned samples were stained with a 0.01 % (w/v) aqueous solution of acridine orange, left for 5 minutes in darkness, and rinsed in water prior to viewing.

2.3.1.1.2 Calcofluor

Plant tissues were stained by mounting in a 0.01 % (w/v) aqueous solution of calcofluor, and were examined under the illumination of the fluorescence microscope.

2.3.1.2 Roots

The procedure for roots was similar to that of leaf, with the exception that:

a) roots were cut at the oldest section developed (near the base of the stem), and

b) were not partitioned in 3 ages and were examined as a thin slice section.

2.4 STOMATAL MEASUREMENTS

To study the microscopic pores of wheat leaves a thin layer of clear nail varnish (Boots No 17) was applied to about a 10 mm^2 area of the wheat leaf adaxial (upper) surface. After allowing the nail varnish to dry (2-3 minutes), it was peeled off with forceps resulting in a replica imprint. The impression was placed onto a microscopic slide with a cover glass, and viewed under a compound microscope at x10 magnification. Measurements were made of stomata and epidermal cell numbers per given area to give Stomatal Density. For the calculation of Stomatal Index, epidermal cell and stomatal cell numbers were counted. For each treatment 3 leaves were sampled and 10 fields of view were counted per sample.

Stomata size, as guard cell length, was determined by the use of an eyepiece unit graticule. The graticule was aligned and calibrated with a stage micrometer, that is scaled 0-1 mm divided into 100 units (i.e. 1 unit = 10 μm).

Stomata Density (SD) was calculated as:

$$SD = \frac{S}{Area}$$

Stomatal Index (*SI*) was calculated according to Salisbury's (1927,1932) equation:

$$SI = (\frac{S}{E + S}) \times 100$$

S = number of stomata, E = number of epidermal cells, and Area = area of field of view.

2.5 CHLOROPHYLL EXTRACTION AND ESTIMATION.

In order to isolate chlorophyll pigments from the wheat fourth leaf, 0.2 g fresh weight of tissue was chopped into small pieces, placed in a test tube with 10 ml of 80 % (v/v) acetone. The test tubes were then sealed with Parafilm and incubated overnight in darkness, at room temperature. Next day, the solutions were removed from the test tubes, placed in a 1 cm glass cuvette, and the light absorption was recorded in a Unicam SP800 spectrophotometer.

In this technique, the amount of light that chlorophyll a, and b absorbed, measured at wavelength of 645 nm and 663 nm, was used to determine the chlorophyll concentration in a sample (based on a gram dry weight) by using the equations of Holden (1976):

One. Chlorophyll a (mg/l) = (12.7 X A663) - (2.69 X A645)
Two. Chlorophyll b (mg/l) = (22.9 X A645) - (4.68 X A663)
Three. Total Chlorophyll (mg/l) = (20.2 X A645) + (8.02 X A663)

2.6 PROLINE ESTIMATION

The method of Bates *et al.*, (1973) was used originally but it was not possible to detect proline. Therefore an alternative method was used.

To demonstrate the proline accumulated by the wheat fourth leaf, the following procedure was adopted, modified from the method used by Singh *et al.*, (1973) for amino acid determination. Total soluble amino acids were extracted from 0.5 g fresh leaf tissue homogenised in a pestle and mortar in 2 ml of methanol:chloroform:water (M:C:W) in the ratio of 12:5:1 (v/v/v). The homogenate was centrifuged at 6000 rpm for 10 minutes in a bench top centrifuge then the supernatant was collected and retained. The residue was then shaken for 5 minutes with 2 ml of M:C:W and centrifuged again as above. The supernatants were mixed together and formed a stable emulsion. To break the emulsion 1.5 ml of distilled water and 1 ml of chloroform were added individually to the mixture resulting in the formation of two layers. The upper layer formed was a methanol-water phase which contained the amino acids, and the lower layer was chloroform containing chlorophyll, as represented in Figure 1.

2.6.1 Thin Layer Chromatography (TLC)

The upper phase of the broken emulsion (amino acid extraction) was taken out in 10 μl portions into small volume tubes, and were stored if necessary. Samples of the solution were applied to pre-formed aluminium TLC plates, 20 X 20 cm in size, made of silica gel at a layer thickness of 0.2 mm. The TLC plates was supplied by Sigma company. In this part of the experiment, two separate developing solvent systems were used:

a). One solvent system (one dimension): In this way seven, evenly-spaced marks 2.5 cm apart, 3 cm from the edges, and 3 cm from the bottom of the TLC plate were arranged on the horizontal axis. Solution samples of 5, 30, 35, 40, 45, 50, and 10μl were applied to the indicated marks. Marks number 1 and 7 were used for standard L-proline samples, and the other 5 marks used for the unknown mixture. The TLC plate was then developed in a developing solvent of 95% ethanol (EtOH):water ratio 5:1 (solvent 1).

b). Two solvent system (two dimension): In this case, only 30, or 50μl of the unknown amino acid solution was applied to a chromatogram plate. The spot was marked 3.5 cm from right edge, and 3 cm from the base of the plate. The first development of the TLC plate was in solvent 1. The TLC plate was removed and left to air dry. Secondly, it was rotated to a right angle from the first development then was inserted into solvent 2: chloroform:methanol:17 % ammonium hydroxide in the ratio of 2:2:1. In order to localise proline in this system it was necessary to run replicate plates of pure proline solution and also solutions of extracted amino acids to which excess proline was added.

In both systems, chromatogram plates were removed from the solvent when the solvent front was about 3-4 cm from the top of the plate and allowed to dry in air. To detect the proline spots, the plates were lightly and evenly sprayed with commercially prepared Ninhydrin spray, and heated in a drying oven at 110 C for 10 minutes. In addition, to preserve the chromatograms TLC plates were uniformly sprayed with commercial Ninhydrin fixer until the background became light blue. The fixer was neutralised immediately while still wet by spraying with Ammonia spray reagent. TLC plates were scanned using a Logitech page colour scan and the image of

PROLINE EXTRACTION DIAGRAM

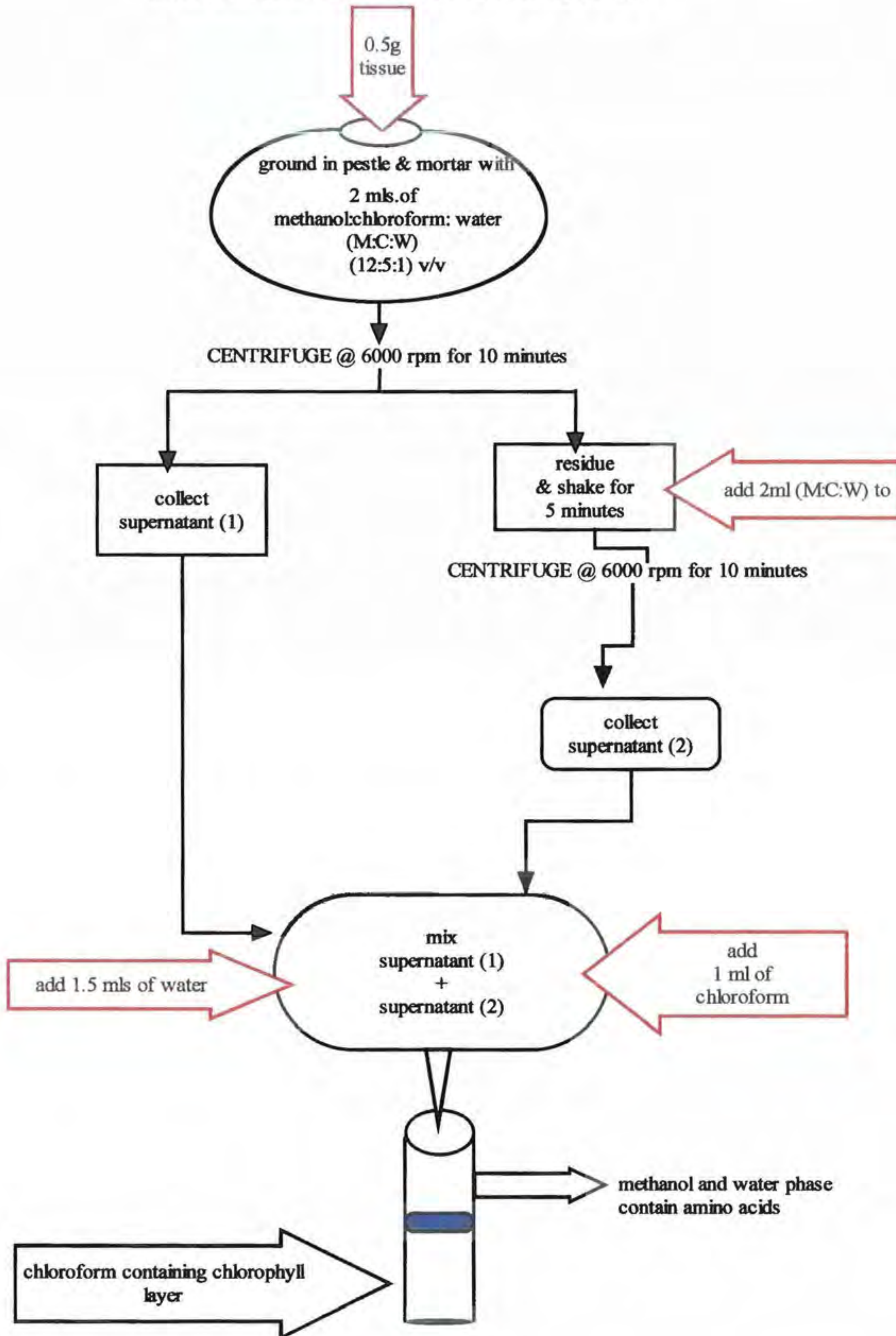


Figure 1

the amino acid spots were enhanced using the Adobe Photoshop paint function. R*f* values were measured and recorded from printed images. All chemical spray reagents were supplied by Sigma.

2.7 STATISTICAL ANALYSES

All data were analysed using two-way analysis of variance for repeated measurements, results are expressed as mean ± SEM (unless stated other wise). The statistical analyses were carried out using GraphPad Prism software (version 2.0, GraphPad, 1995) on IBM compatible computer. Statistical significance was deemed when P value is less than 0.05 ($P \leq 0.05$).

RESULTS

SECTION I: POT EXPERIMENTS

3.1.1 Initial Observations

In preliminary experiments, it was observed that pre-treatment of wheat caryopses with Cycocel allowed seedlings derived from them to survive longer than control plants under conditions of induced drought stress. For example the application of 0.4 % Cycocel to the caryopses of wheat resulted in an increase of tolerance of the plants whereby they remained turgid and green for about 23 days after water was withheld (Plate 1). Importantly the seedlings were able to re-initiate growth when water was supplied after this period.

In contrast, the first sign of incipient wilting in untreated wheat plants started at about 7 days after the cessation of last watering, and by about the 12th day these plants were completely lodged and dry. These plants did not recover on re-watering at day 23. To further characterise this effects plants were grown from either treated or untreated caryopses up to the 5th leaf stage. They were subjected to a water withholding treatment for about 15 days when the analyses of available water were conducted. At 15 days the plants from treated caryopses were still turgid whilst those from untreated caryopses were wilting and drying (Plate 2).

Plate 1

The effect of pre-treatment of wheat caryopses with Cycocel on the ability of seedlings to withstand water limitation. Caryopses were imbibed in 0.4 % Cycocel or water and seedlings were grown in soil until the 4th leaf was mature. Water was then withheld for 23 days.

Plate 1

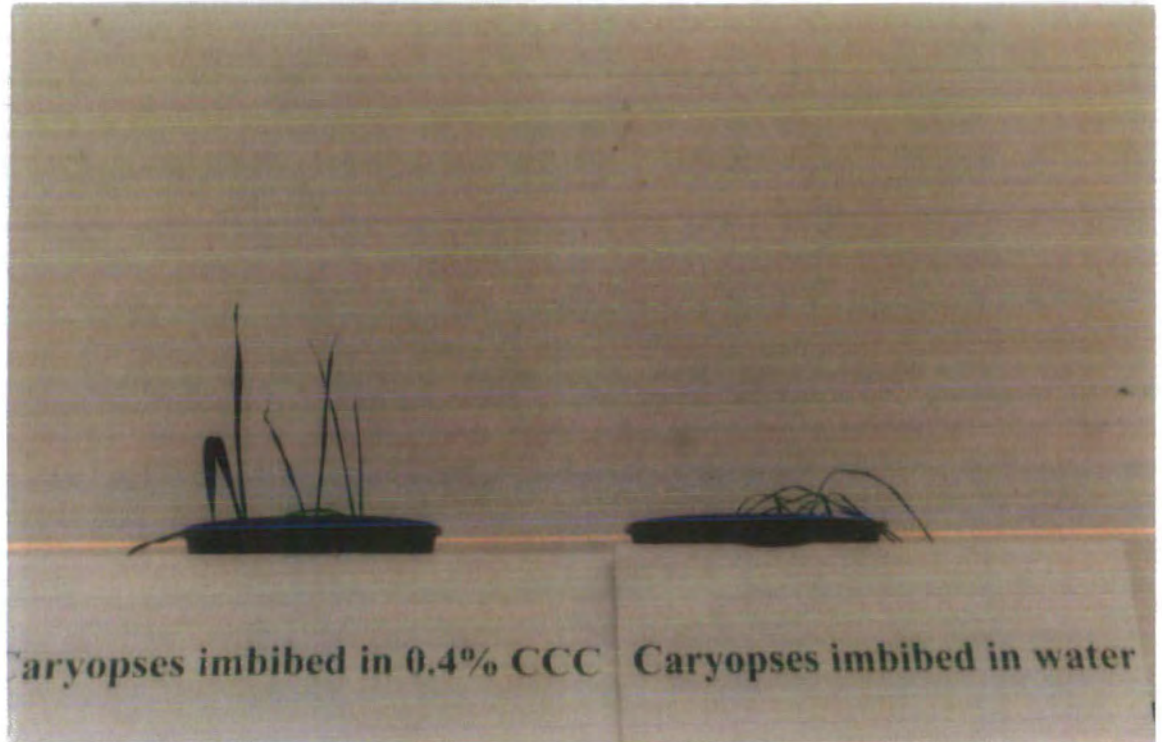
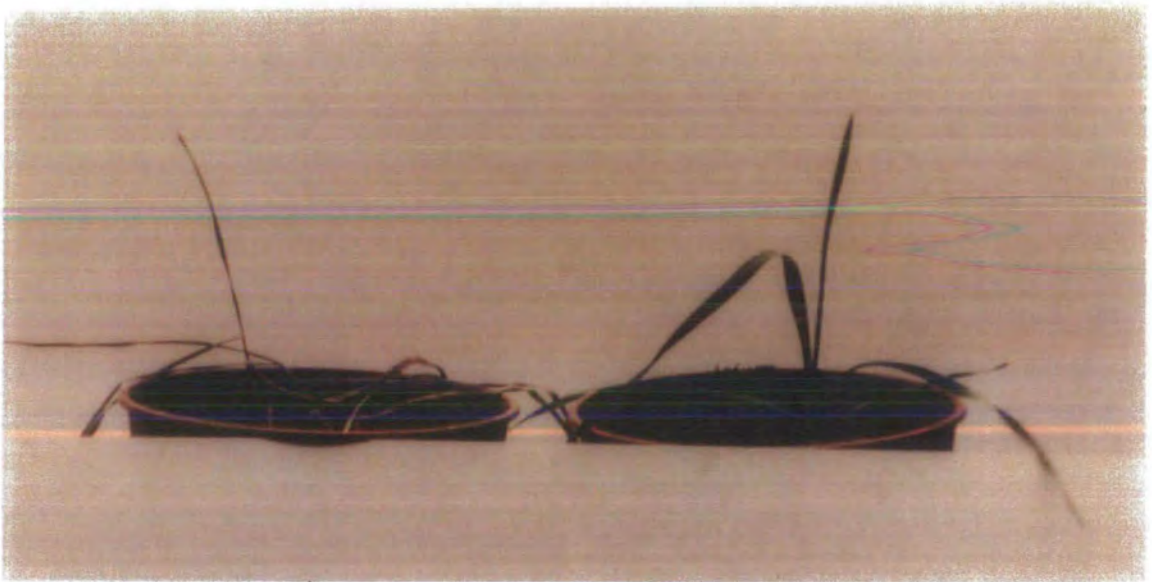


Plate 2

The effect of pre-treatment of wheat caryopses with Cycocel on the ability of seedlings to withstand water limitation. Caryopses were imbibed in 0.4 % Cycocel or water and seedlings were grown in soil until the 4th leaf was mature. Water was then withheld for 15 days.

Plate 2



Caryopses germinated in water. Water withheld for 15 days.

Caryopses germinated in 0.4 % Cycocel. Water withheld for 15 days.

The percent water available in plants after 15 days of water withholding was estimated as follows:

$$\% \text{ water available in plants} = \frac{\text{Water lost from plants after artificial drying}}{\text{Fresh weight of plants at harvest}},$$

It appears that water regime influences the ability to retain water where those plants subjected to moderate drought stress retain more than those which were water regulated. The same pattern was also seen with plants from Cycocel pre-treated caryopses, however, much more water was retained overall in the plants of the treated caryopses (Table 2). The corresponding levels of water in the soil were similar for all the pots. These values compare with the soil at field capacity of 88.65 %. It was also seen that GEN2 plants derived from GEN1 plants grown from treated caryopses showed some enhanced ability to retain water. The same pattern was also evident in the tillers evolved from pre-treated 2-day watering plants.

Apparently the treatment of caryopses with Cycocel gives some protection against water stress in seedlings derived from them. Based on these observation attempts were made to identify the underlying mechanisms for enhanced capacity to withstand the effects of water withholding.

Table 2

The influence of Cycocel, water withholding and the combination of both on the ability of wheat seedlings to retain water after 15 days lack of water.

* 2 days = plants were watered every 2 days until the emergence of 5th leaf.

* 7 days = plants were watered every 7 days until the emergence of 5th leaf.

GEN1 = refer to the plants from commercial wheat caryopses.

2nd = secondary tillers excised from the 1st produced tillers.

3rd = subsequent tillers of the secondary tillers.

Soil = the comparative levels of water in the soils.

Table 2

		2 days *	7 days *
Type	Treatment	% water available	% water available
Plant:			
F1	Control	2.41	13.08
F1	Cycocel	43.36	62.15
Soil			
F1	-	9.65	13.90
F1	-	13.73	13.79
Tillers:			
2nd	-	26.14	
3rd	-	9.21	
Soil			
2nd	-	14.89	
3rd	-	11.08	
Soil at field capacity		88.65	

3.1.2 Morphological Effects of Cycocel on Wheat Seedlings

The effects of the Cycocel on the germination and seedling development of pre-treated caryopses were monitored as a preliminary investigation through the growth of wheat plants.

3.1.3 Germination of Caryopses

The pre-treatment of caryopses (overnight imbibition) with 0.004, 0.04, and 0.4 % aqueous solutions of commercial Cycocel reduced the rate of germination in all cases. The effect was most significant with the application of 0.4 % Cycocel, and this concentration was the one which reduced overall germination percentage after 5 days (Figure 2). At 4 % Cycocel, the germination of wheat caryopses were retarded markedly to the extent that caryopses became infected with fungi which resulted in their death. This range of concentrations was used to determine a suitable treatment level which would have an effect on the plants but not a lethal one, to this extent 0.4 % proved to be an effective concentration.

3.1.4 Seedling Growth

The most prominent feature of all was the development of shorter plants supported by a thicker stem, and a decrease in the length of leaves.

The marked decrease in the length of stem and leaves represented an important feature of the morphological changes associated with the application of the growth retardant Cycocel. This feature was most prominent with the use of 0.4 % Cycocel solution for the pre-treatment of caryopses whilst still allowing the plants to survive (Plate 3).

3.1.5 Effect of Cycocel on Seedling Growth in Combination with Different Watering Regimes

Leaves of plants derived from caryopses germinated in water showed a decrease in overall growth when subjected to a water withholding regime of 7-day watering (Figure 3). No effect was seen on the first leaf, but the growth reduction was highly significant in the second, 3rd, and 4th leaf. The 7-day watering regime was having a physiological effect on the plants and could be used as a treatment to impose mild water stress. This baseline response was used to determine the effect of Cycocel on the growth of wheat seedlings under different watering regimes. The growth of pre-treated wheat seedlings was substantially reduced through the early vegetative production. Therefore the effects of Cycocel and Cycocel + water withholding on leaf length were used as an indicator of the plant growth development. It was observed that under continuous watering and water withholding regimes, a number of Cycocel treatments significantly affected the growth of wheat leaves.

The growth of all successive leaves, up to the 4th , was reduced by Cycocel treatment under both water regimes (Figure 4 & 5), While the same concentrations, however, suppressed the growth of the wheat leaves under a continuos watering regime. The conspicuous effect was on the 4th leaf of plants which were watered under the 7-day regime. Here this leaf did not develop in seedlings for caryopses treated with 0.004 and 0.04 % Cycocel but did at higher concentration of 0.4 %. Also these leaves were enhanced in their growth compared with those of plants set under the 2-day watering regime but their growth was still reduced in relation to the growth of comparable leaves of the control plants.

Figure 2

Rate of germination of wheat caryopses following Cycocel treatment. Caryopses of wheat were soaked overnight and germinated either in water or in a concentration of commercial Cycocel. The Cumulative percent germination was recorded daily.
30 seeds were sown into 5 replicate Petri-dishes for each treatment; germination was recognised when the radicles protruded for the caryopses.

Figure 2

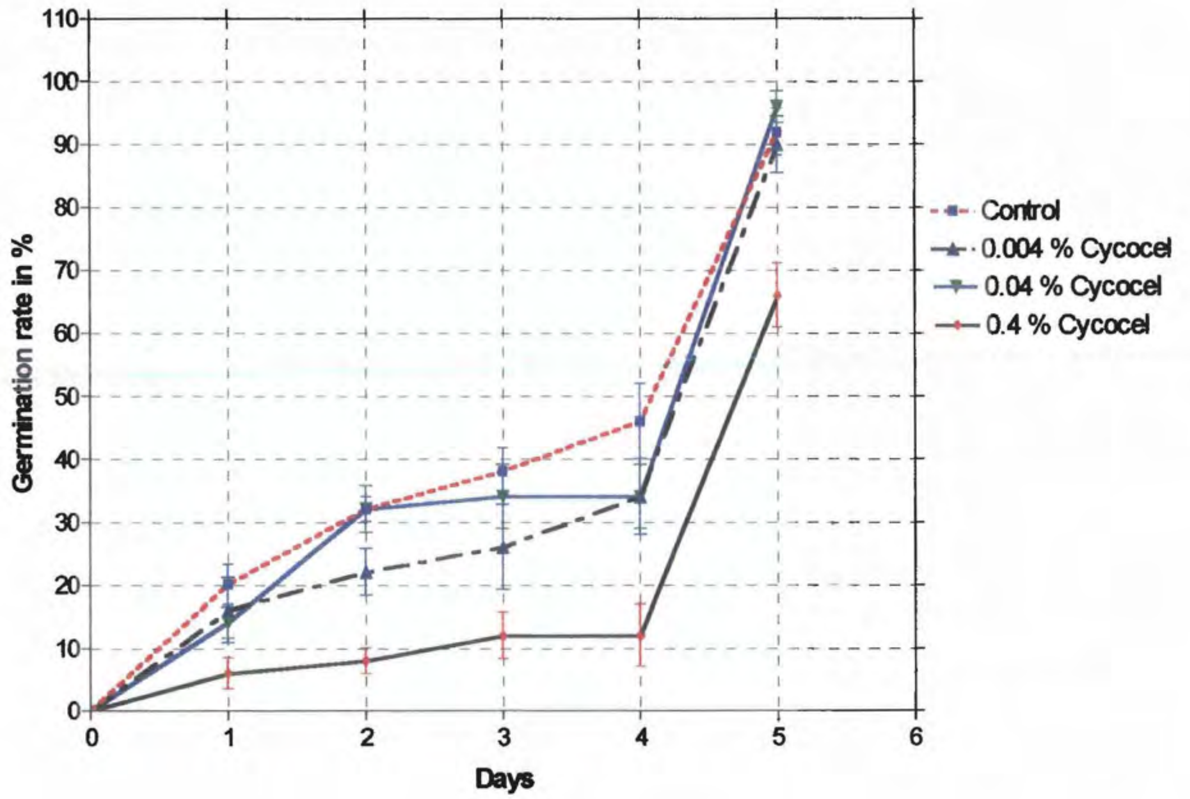


Plate 3

Effect of pre-treatment of wheat caryopses with Cycocel on the growth of seedlings.

Figure 3

Length of individual leaves of wheat seedlings from caryopses germinated in water alone. Plants were subjected to 2-day and 7-day watering regimes.

Plate 3

Caryopses germinated in water

Caryopses germinated in 0.4 % Cycocel

Figure 3

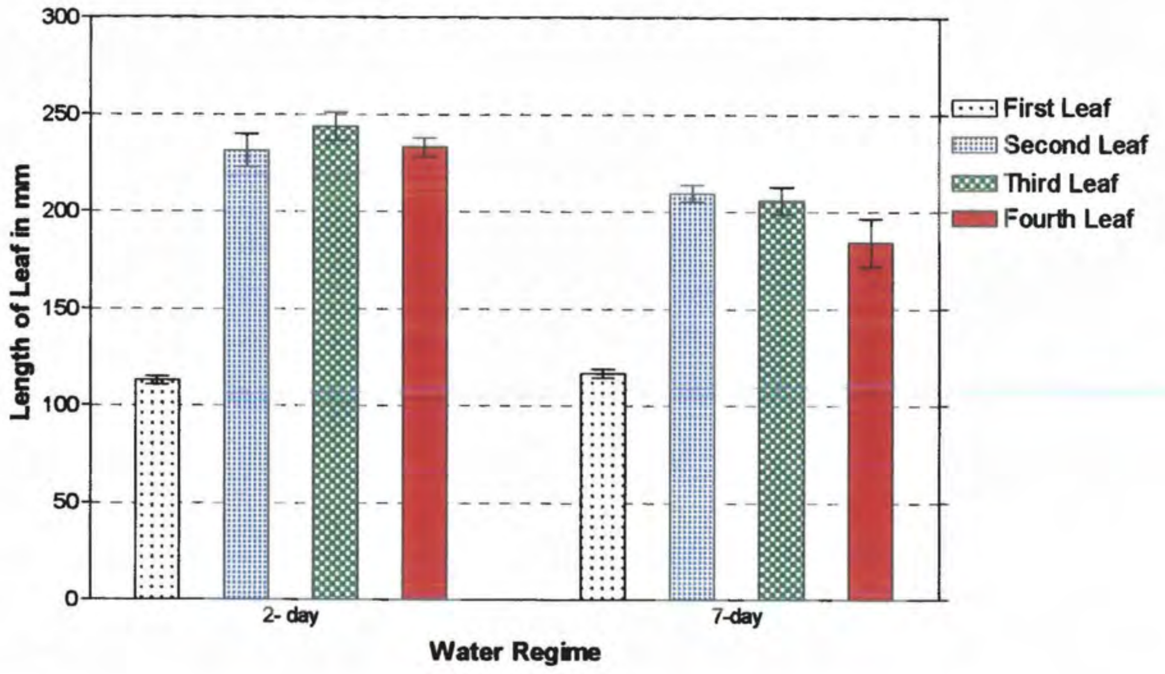


Figure 4

Effects of Cycocel concentration on the growth of individual wheat leaves grown under a 2-day watering regime in a controlled environment growth room.

Figure 5

Effects of Cycocel concentration on the growth of individual wheat leaves grown under a 7-day watering regime in a controlled environment growth room.

Figure 4

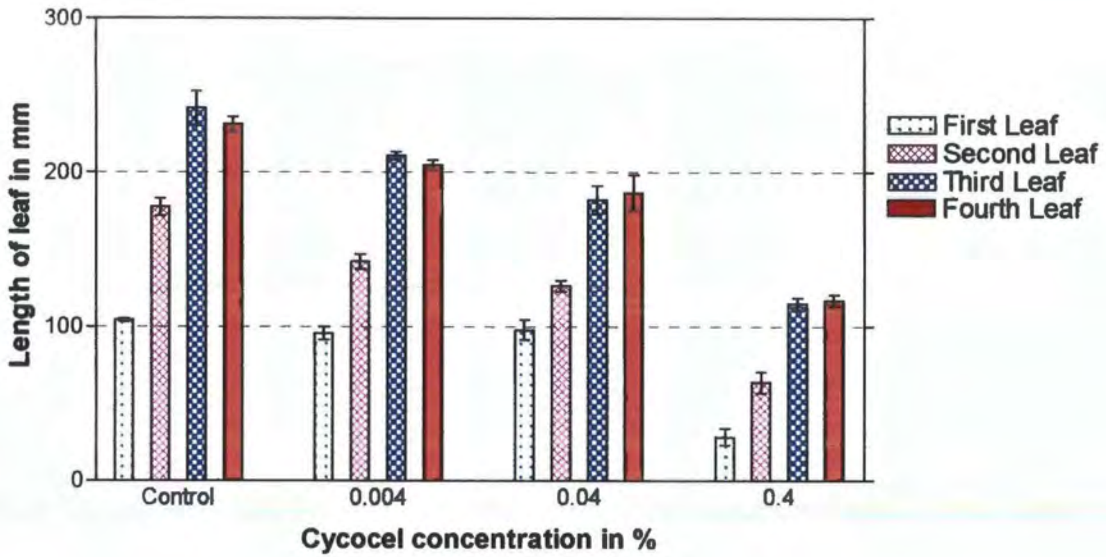
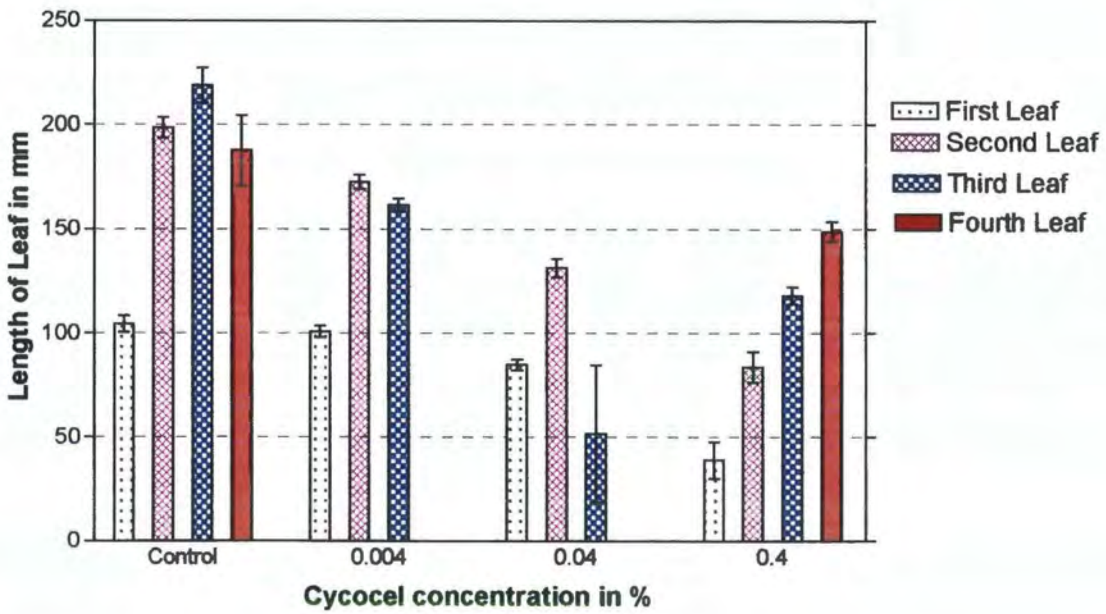


Figure 5



3.1.6 Pigmentation

Interestingly, the effect of Cycocel was apparent in decreasing the size of wheat leaves, however, the size seems to be compensated for by a darker green appearance of the leaves. The influence of Cycocel on the greening process of the individual wheat leaves, up to the 4th leaf, was investigated, and chlorophyll accumulation was measured under two different watering regimes. The influence of Cycocel on total chlorophyll accumulation is shown in Figure 6 (2-day) and Figure 7 (4-day).

Overall, it appears that as the leaves age, the content of chlorophyll per g dry weight decreases, under 4-day watering regime the level of chlorophyll is reduced in all leaves compared with the 2-day watering regime. Treatment with 0.04 % Cycocel gave a significant increase of chlorophyll levels in all leaves except the 4th.

Cycocel treatment also increased the chlorophyll levels over the control values under the 4-day watering regime (Figure 7). However, as with the controls the overall level of chlorophyll was reduced. Cycocel appeared not to counter the reduction in chlorophyll content seen in response to the watering regime.

Individual contents of chlorophyll a and chlorophyll b were also determined. The results are shown in figures. 8, 9, 10, and 11. The effect of watering regime itself and in combination with Cycocel treatment was essentially the same for the chlorophyll a and chlorophyll b as for the total chlorophyll. No differential effect was seen on the two photosynthetic system pigments.

Pre-treatment of wheat caryopses resulted in distinct morphological effects on wheat seedlings derived from them. Whilst reduced in size the seedlings are able to grow and have enhanced capacity to withstand the effects of induced water stress.

Figure 6.

The influence of pre-treatment of caryopses with Cycocel on the total chlorophyll accumulation in wheat leaves, when grown under a 2-day watering regime.

Figure 7.

The influence of pre-treatment of caryopses with Cycocel on the total chlorophyll accumulation in wheat leaves, when grown under a 4-day watering regime.

Figure 6

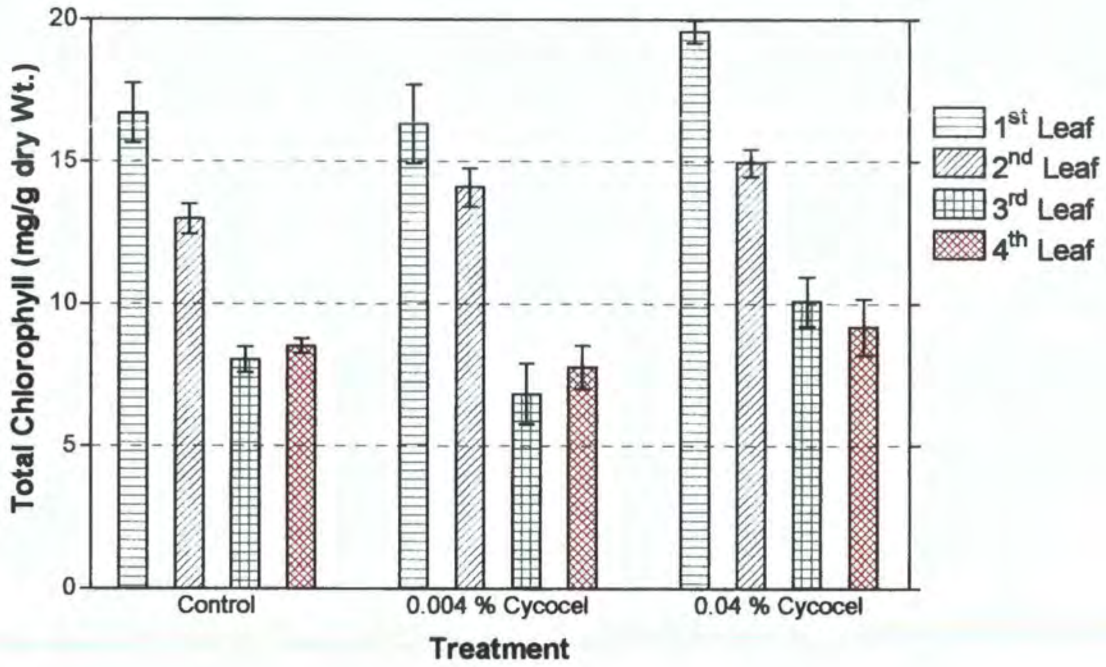


Figure 7

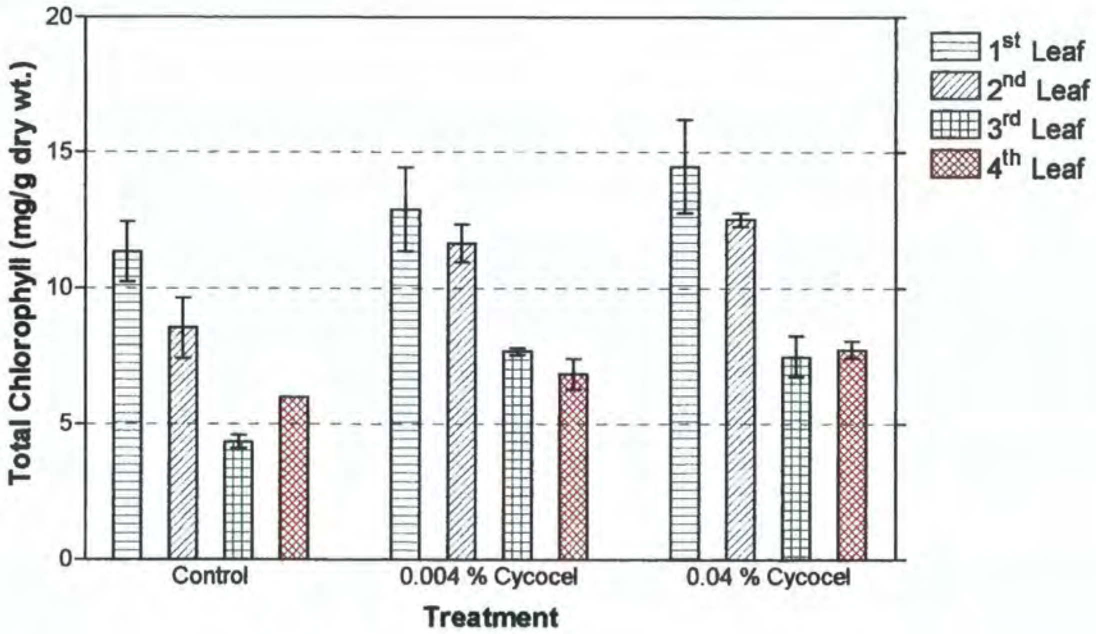


Figure 8 & 9.

The influence of pre-treatment of caryopses with Cycocel on chlorophyll a and chlorophyll b accumulation in wheat leaves, when grown under a 2-day watering regime.

Figure 8

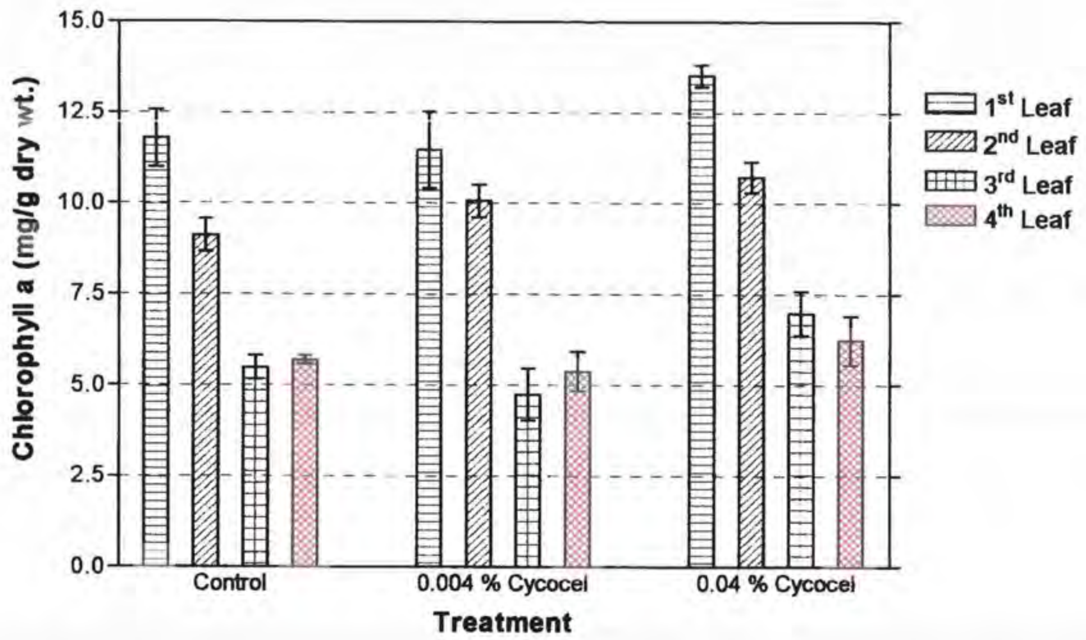


Figure 9

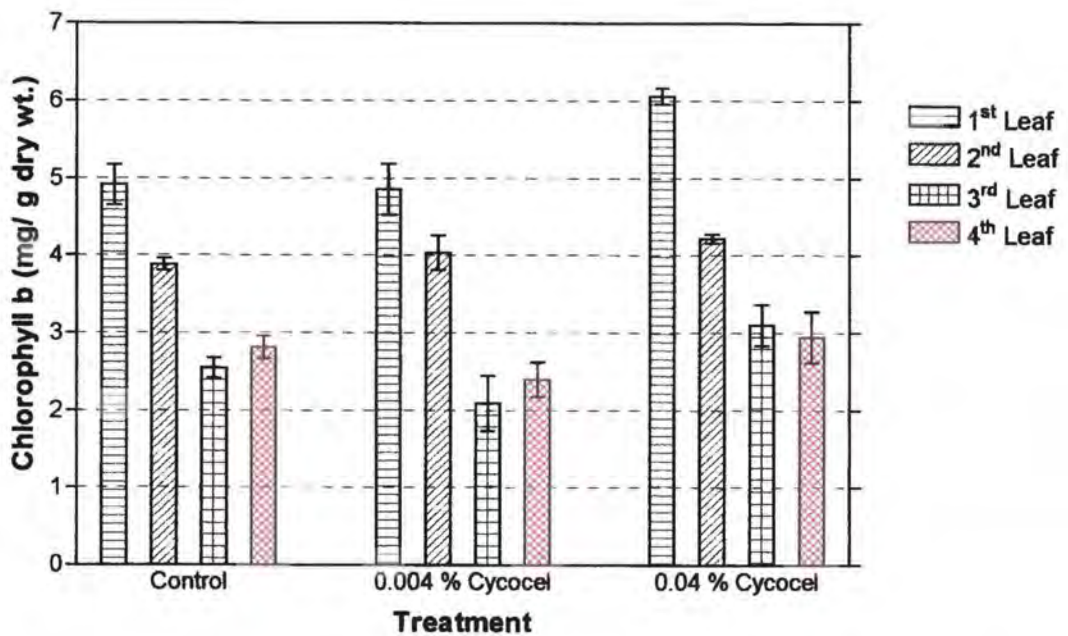


Figure 10 & 11.

The influence of pre-treatment of caryopses with Cycocel on chlorophyll a and chlorophyll b accumulation in wheat leaves, when grown under a 4-day watering regime.

Figure 10

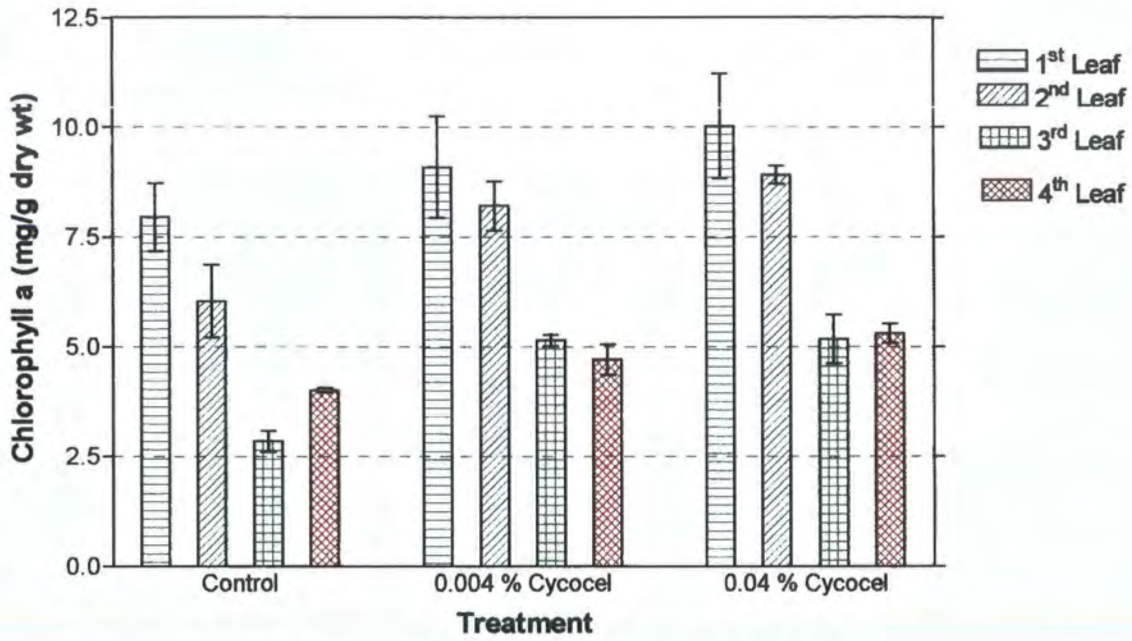
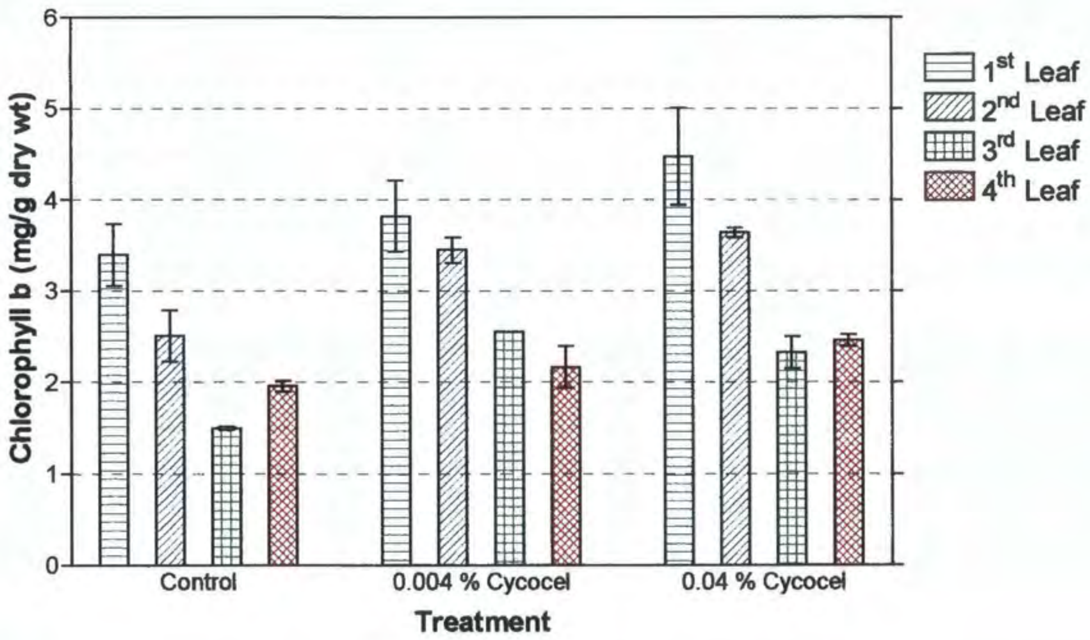


Figure 11



3.1.7 Proline Accumulation

The accumulation of compatible solutes under water stress conditions has been demonstrated for many plants. Wheat has been shown to accumulate proline under these conditions. An investigation was undertaken to determine whether or not Cycocel pre-treatment of caryopses modified the ability of wheat seedlings to accumulate proline as a mean of tolerating water stress.

Wheat leaf samples were originally analysed for proline using the method of Bates *et al.*, (1973) but it was not possible to detect proline by this method even when proline was added to the extracted sample. Therefore an alternative procedure of Thin Layer Chromatography (TLC) method was adopted and modified from that used by Sing *et al.*, (1973) for amino acid determination.

The results for the analysis of the extracted total amino acids showed no accumulation of proline under a 2-day watering regime for the plants derived from caryopses germinated in water or Cycocel (Figure 12 and 13). This result suggests primarily that Cycocel, under a continuous watering regime, has no influence directly on the proline accumulation.

However, one noticeable difference between the samples was the presence of two purple-staining bands in the amino acid derived from control tissue at R*f* approximately 0.62 and 0.71 compared with one band in the Cycocel-treated material at R*f* 0.73. The identity of these bands was not established but from their staining properties with Ninhydrin they were assumed to be amino acids. Some slight variation in R*f* values of various bands from the same samples were also detected (Table 3).

As can be seen from Figure 14 the presence of proline in a total amino acid extraction can be detected by this method. Here a sample of standard proline was

added to a total amino acid extraction from wheat leaves, under normal watering conditions, prior to analyses by TLC.

Plants which were subjected to a 7-day watering regime, and were derived from caryopses treated with Cycocel, accumulated proline (Figure 15). Likewise proline was detected in plants which were deprived of water from 20 days prior to extraction (Figure 16). *Rf* values again for the components are seen in Table 4. In both cases, however, it was not possible to extract the plants derived from caryopses germinated in water since they did not survive the water withholding conditions.

These results suggest that normal accumulation of proline can take place in wheat plants in response to water stress. However, it is possible that Cycocel pre-treatment conditions the plants for the production of this amino acid whilst not having a direct effect on proline accumulation. Proline accumulation is potentially initiated once the stress treatment is applied, plants are pre-conditioned to respond by the Cycocel pre-treatment.

Figure 12

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

Sample :-

30 - 50 μl of total amino acids from plants held under a 2-day watering regime, derived from caryopses germinated in water.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 12

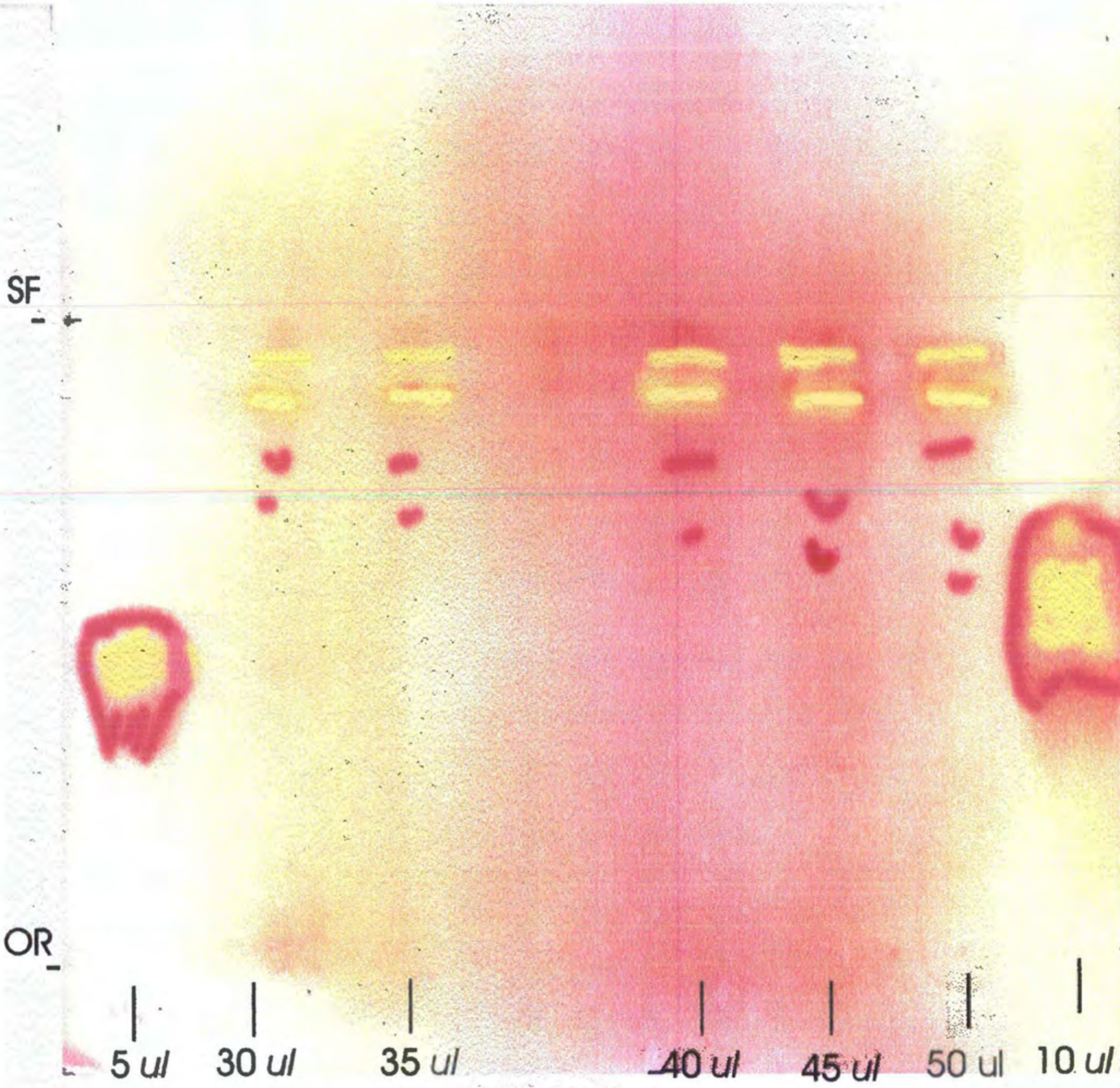


Figure 13

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

Sample :-

30 - 50 μl of total amino acids from plants held under a 2-day watering regime, derived from caryopses germinated in 0.4 % Cycocel.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 13

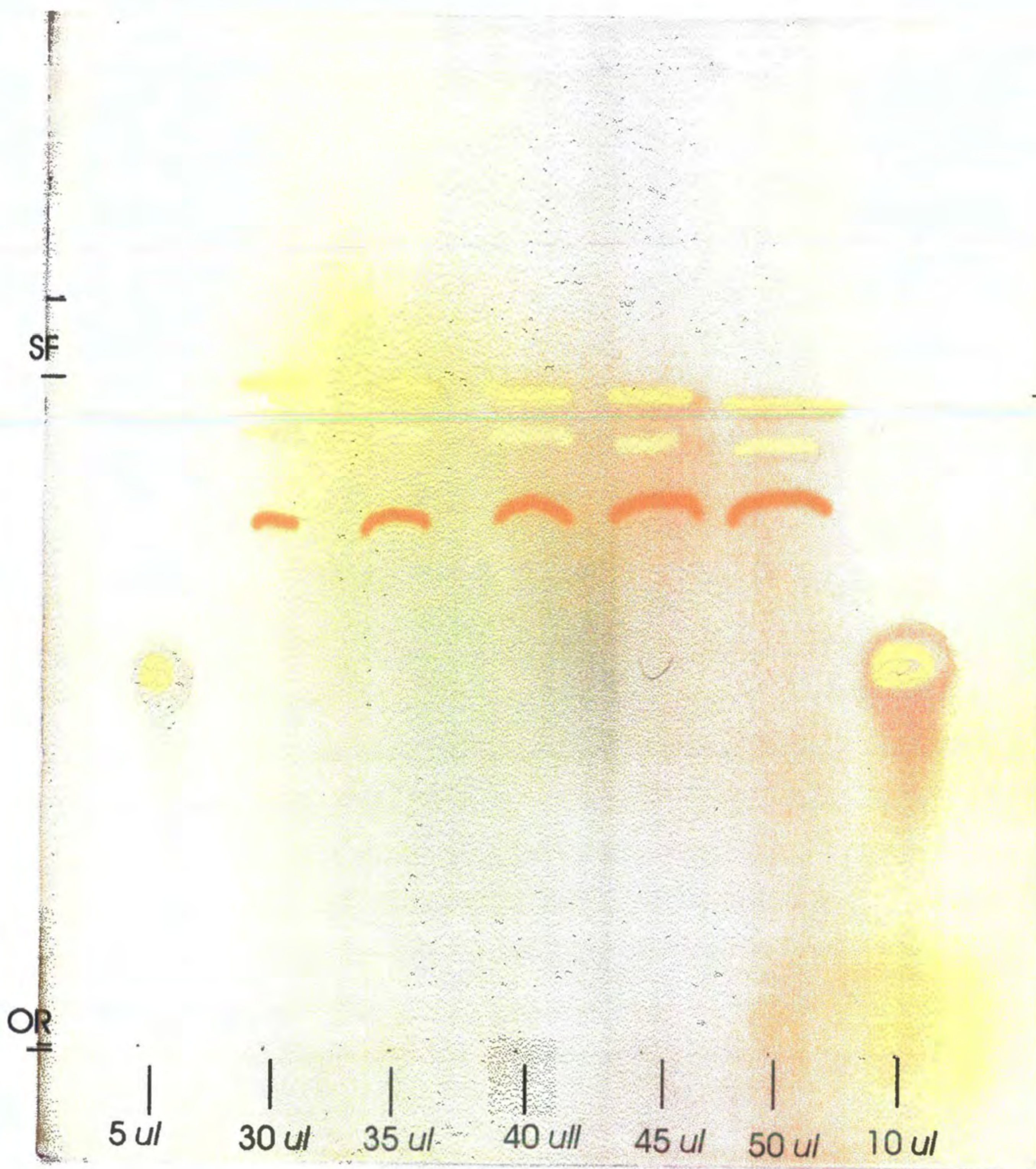


Figure 14

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

Sample :-

30 - 50 μl of total amino acids from plants held under a 2-day watering regime, derived from caryopses germinated in Cycocel. Approximately 1 mg of L-proline was added to the sample before loading.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 14

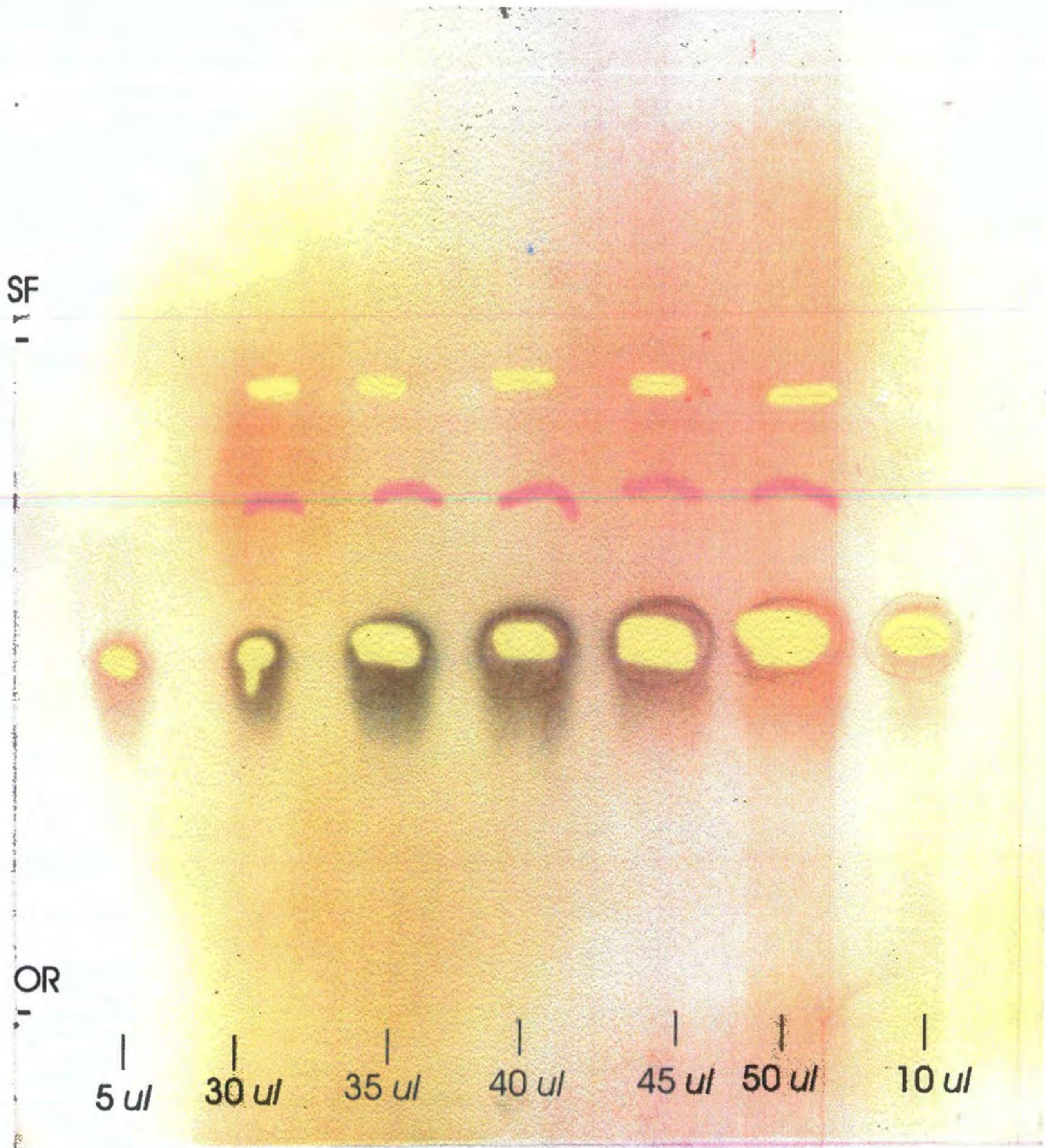


Table 3.

The Rf - values of proline and amino acids bands extracted from wheat leaf tissue.

Control:-

plants from wheat caryopses germinated in water.

Treated;-

plants from wheat caryopses germinated in 0.4 % Cycocel.

Visible pigments was seen before spraying with Ninhydrin.

Table 3

Treatment	Watering regime	Concentration *ul* [1]	Proline	Colour of spot	Unknown band	Colour of spot	Unknown band	Colour of spot	visible Pigment	Colour of spot	visible Pigment	Colour of spot
Control			R*f*		R*f*		R*f*		R*f*		R*f*	
	2 days	*5 ul*	0.45	Yellow								
		30 ul			0.66	Light Pink	0.76	Light Pink	0.86	Yellow	0.91	Yellow
		35 ul			0.66	Light Pink	0.76	Light Pink	0.86	Yellow	0.91	Yellow
		40 ul			0.62	Light Pink	0.71	Light Pink	0.86	Yellow	0.92	Yellow
		45 ul			0.60	Light Pink	0.68	Light Pink	0.86	Yellow	0.92	Yellow
		50 ul			0.56	Light Pink	0.62	Light Pink	0.85	Yellow	0.91	Yellow
		10 ul	0.58	Yellow								
Treated												
	2 days	*5 ul*	0.50	Yellow								
		30 ul					0.71	Light Pink	0.84	Yellow	0.89	Yellow
		35 ul					0.71	Light Pink	0.84	Yellow	0.88	Yellow
		40 ul					0.74	Light Pink	0.82	Yellow	0.88	Yellow
		45 ul					0.74	Light Pink	0.82	Yellow	0.88	Yellow
		50 ul					0.75	Light Pink	0.81	Yellow	0.88	Yellow
		10 ul	0.52	Yellow								

Figure 15

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

Sample :-

30 - 50 μl of total amino acids from plants held under a 7-day watering regime, derived from caryopses germinated in 0.4 % Cycocel.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 15

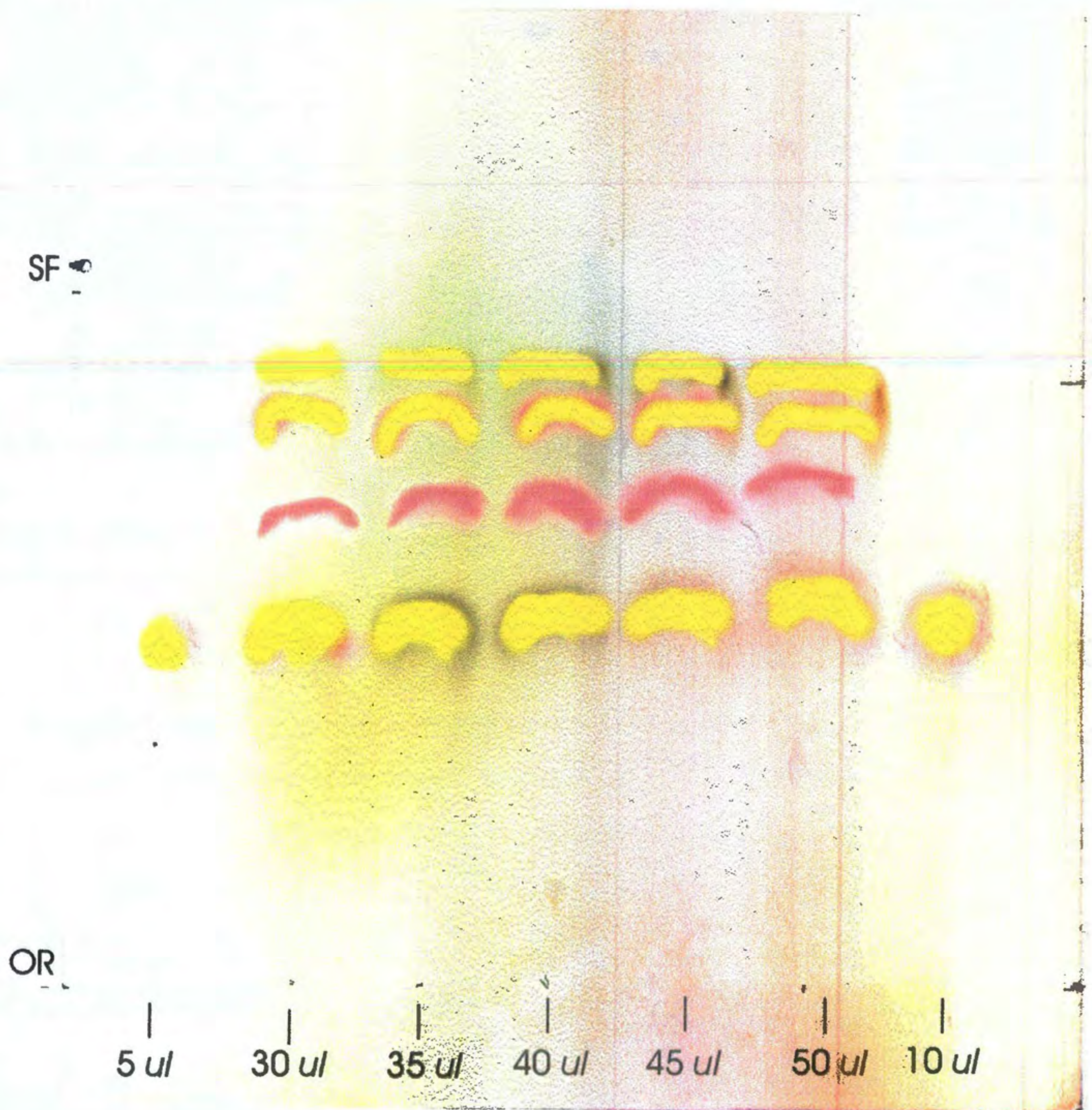


Figure 16

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

Sample :-

30 - 50 µl of total amino acids from plants from which water was withheld for 20 day, derived from caryopses germinated in 0.4 % Cycocel.

Marker:-

5 and 10 µl of standard L-proline solution (outside tracks).

Figure 16

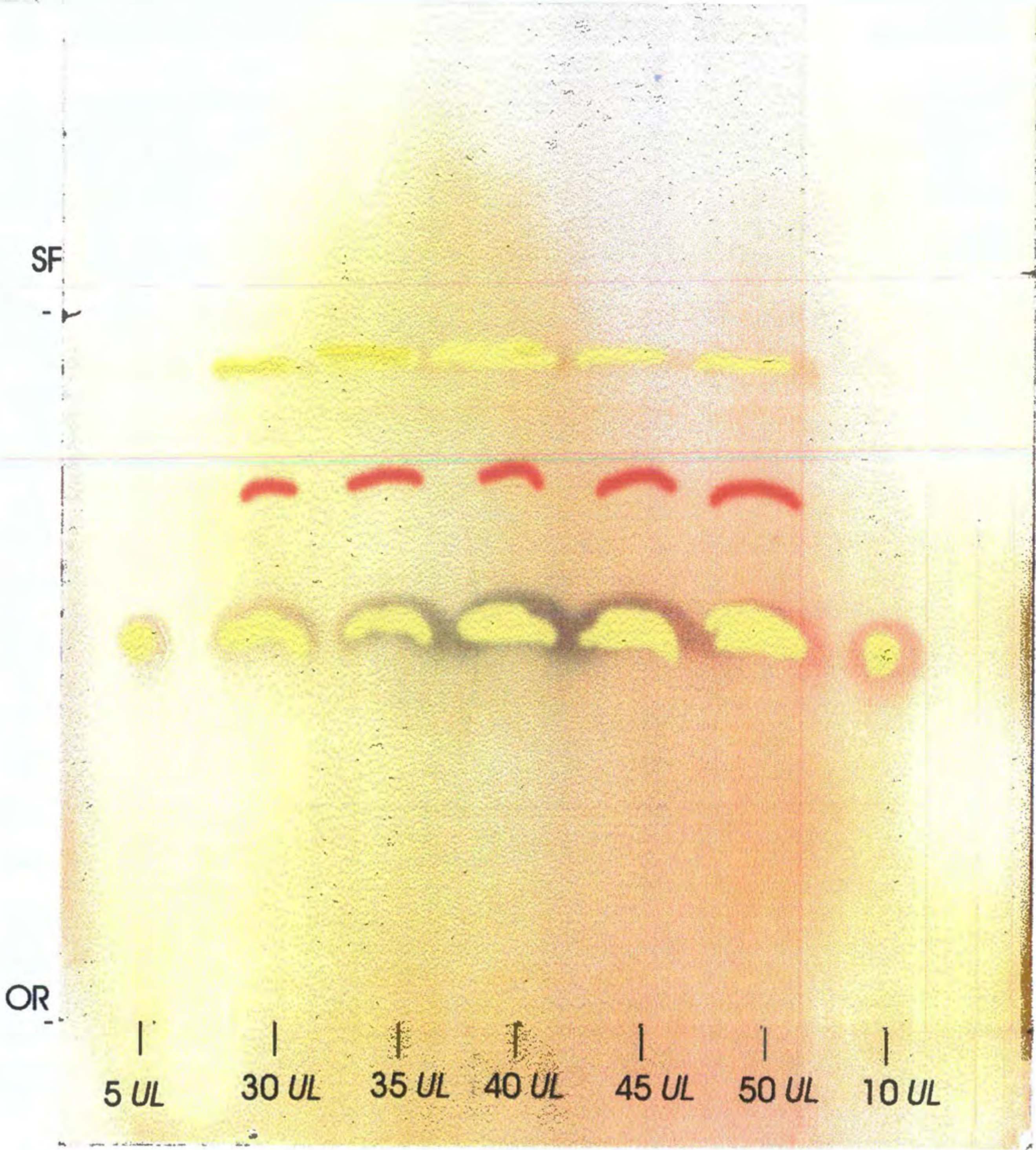


Table 4.

The R*f* - values of proline and amino acids bands extracted from wheat leaf tissue.

Control:-

plants from wheat caryopses germinated in water.

Treated;-

plants from wheat caryopses germinated in 0.4 % Cycocel.

Visible pigments was seen before spraying with Ninhydrin.

* Plants from which water was withheld for 20 days.

Table 4

Treatment	Water regime	Concentration *ul*	Proline	Colour of spot	Unknown band	Colour of spot	visible Pigment	Colour of spot	visible Pigment	Colour of spot
Treated			*Rf*		*Rf*		*Rf*		*Rf*	
	7 days	*5 ul*	0.51	Yellow						
		30 ul	0.54	Yellow	0.71	Light Pink	0.85	Yellow	0.94	Yellow
		35 ul	0.55	Yellow	0.74	Light Pink	0.84	Yellow	0.94	Yellow
		40 ul	0.55	Yellow	0.74	Light Pink	0.85	Yellow	0.94	Yellow
		45 ul	0.55	Yellow	0.75	Light Pink	0.85	Yellow	0.94	Yellow
		50 ul	0.55	Yellow	0.77	Light Pink	0.85	Yellow	0.93	Yellow
		10 ul	0.55	Yellow						
	Stressed *	*5 ul*	0.50	Yellow						
		30 ul	0.56	Yellow	0.71	Light Pink	0.83	Yellow	0.85	Yellow
		35 ul	0.57	Yellow	0.74	Light Pink	0.83	Yellow	0.86	Yellow
		40 ul	0.57	Yellow	0.74	Light Pink	0.83	Yellow	0.86	Yellow
		45 ul	0.57	Yellow	0.73	Light Pink	0.82	Yellow	0.88	Yellow
		50 ul	0.57	Yellow	0.72	Light Pink	0.83	Yellow	0.87	Yellow
		10 ul	0.50	Yellow						

3.1.8 Stomata

In order to try and explain the ability of seedlings derived from Cycocel treated caryopses to tolerate water stress treatments an investigation of the influence of Cycocel on stomatal characteristics was initiated. The influence of Cycocel on stomatal size and frequency, as well as on the epidermal cells frequency was investigated.

Since stomatal closure is one of the profound adaptive mechanism in plants to prevent water loss, the possibility was investigated that prolonged wheat survival during water withholding may have been in response to the changes in stomata characteristics mediated by a pre-treatment of Cycocel. Therefore a preliminary experiment of the effect of the growth retardant on the 3rd, and 4th leaf of wheat seedling during two watering interval regime were conducted. Two preparations of Cycocel were used in this study and were compared.

3.1.9 The effect of Cycocel as Cyanamid

It was found that Cyanamid treatment, at 0.4 %, of caryopses did not alter the length of the stomatal pore present in the seedling leaves (Figure 17). Likewise no change was seen with watering regime alone (Figure 18).

The overall response of Stomatal Density was found to be not significant, Cyanamid did not alter Stomatal Density consistently. The patterns of stomata of the 3rd, and 4th leaf, however, did show some inconsistency of response. Cyanamid treatment resulted in a significant decrease in Stomatal Density in the 3rd leaf tissue (Figure 19). Figure 20 indicates that Cyanamid application appeared to result in a significant Stomatal Density increase under the 4-day watering regime but not at the

other time intervals. Therefore, treatment did not appear to give a significant change consistently. Similar inconsistency was also found in 3rd leaf epidermal cell density (Figure 21), while Figure 22 indicates that the epidermal cells in 4th leaf appear to have been present in equal numbers of cells per mm^2 at all cases, thus the size of epidermal cells was not changed by Cyanamid treatment.

A slightly inconsistent variation in response to Cyanamid treatment was seen also for Stomatal Index (Figure 23 & 24). Cyanamid treatment resulted in a small increase in the Stomatal Index in 3rd leaf of the 7-day water regime plants. However, a similar response was not seen for the 4th leaf (Figure 24). It appears therefore that Cyanamid treatment does not result in changes on stomatal characteristics which would be consistent with a reduction in water loss from the leaves.

Figure 17

Stomatal pore length in μm, as seen in the 3rd leaf of wheat seedlings.

Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 17

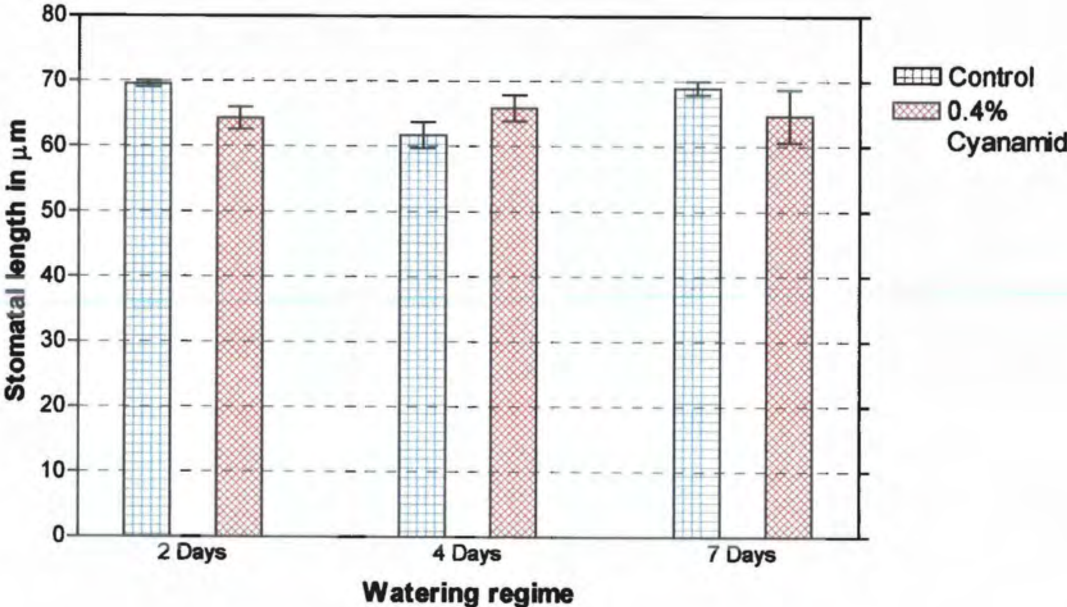


Figure 18

Stomatal pore length in µm, as seen in the 4th leaf of wheat seedlings.

Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 18

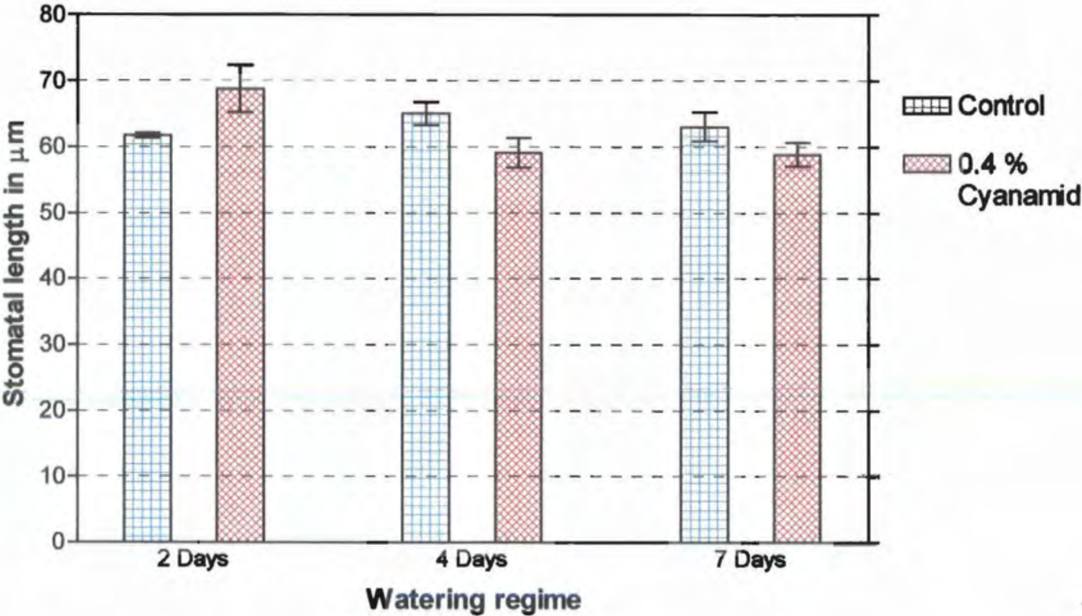


Figure 19

Stomatal Density measured as stomata/mm^2, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 19

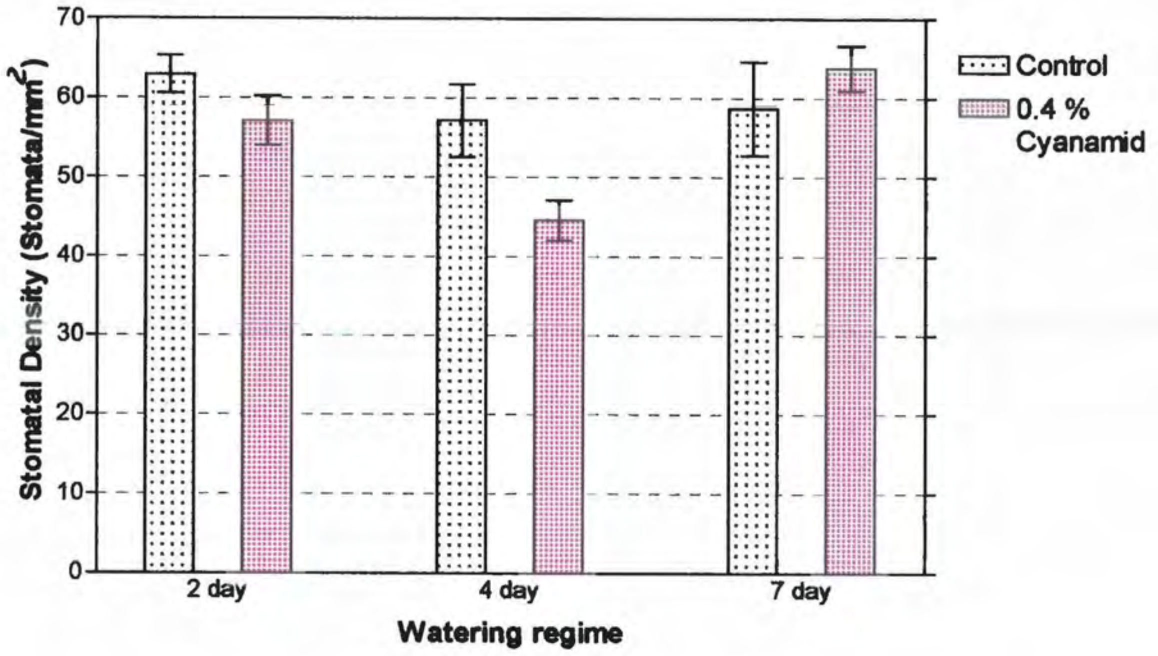


Figure 20

Stomatal Density measured as stomata/mm^2, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 20

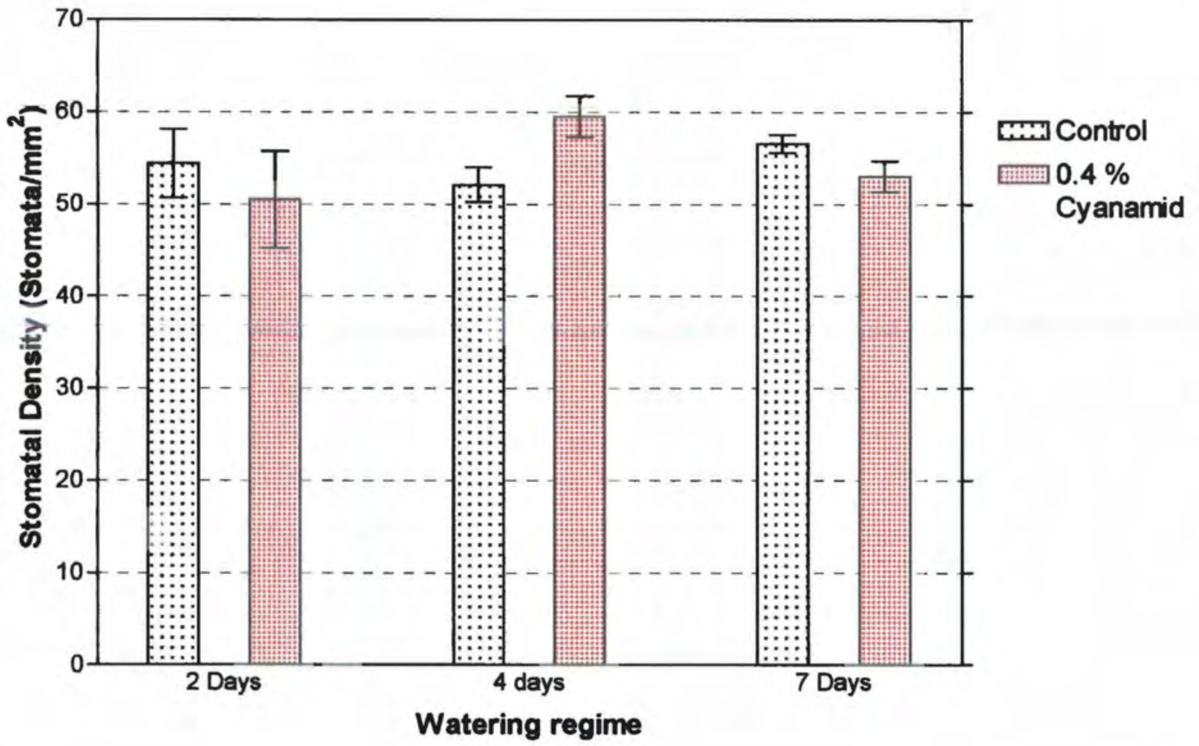


Figure 21

Epidermal cells density measured as epidermal cell/mm^2, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 21

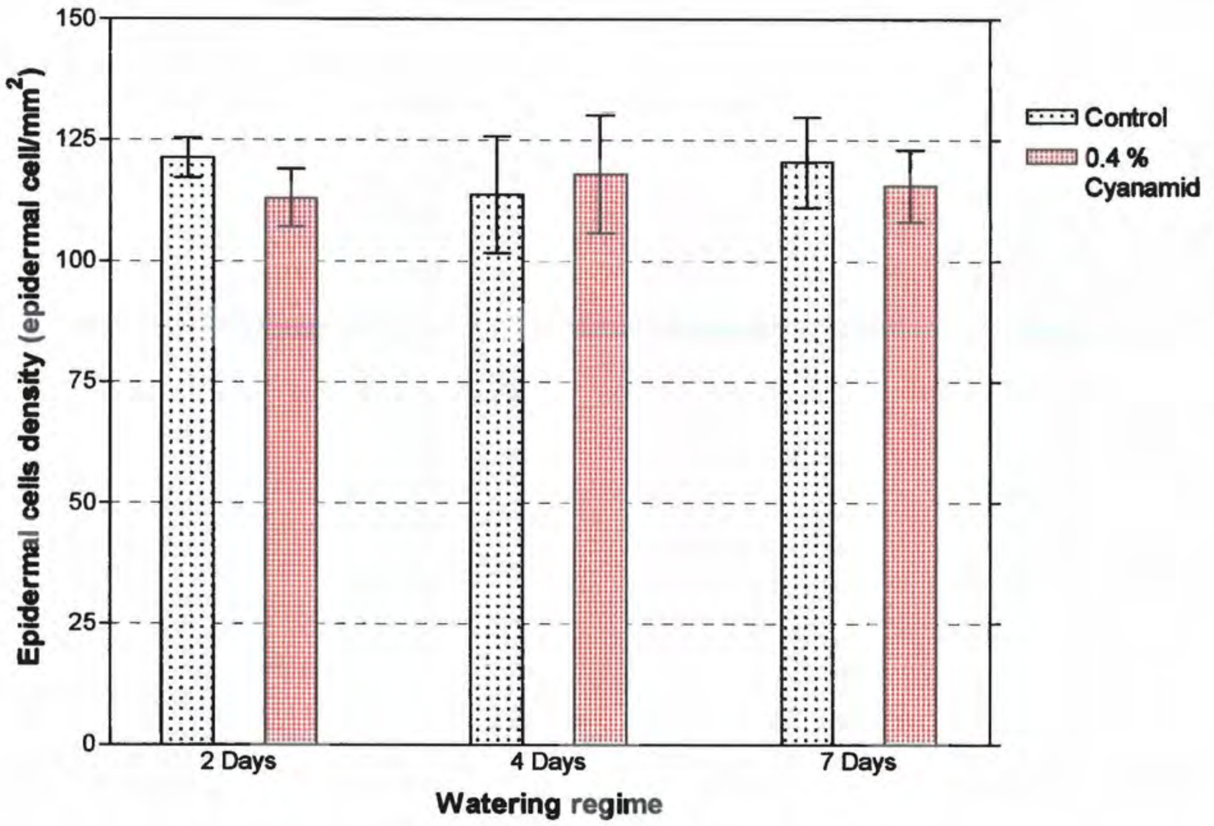


Figure 22

Epidermal cells density measured as epidermal cell/mm^2, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 22

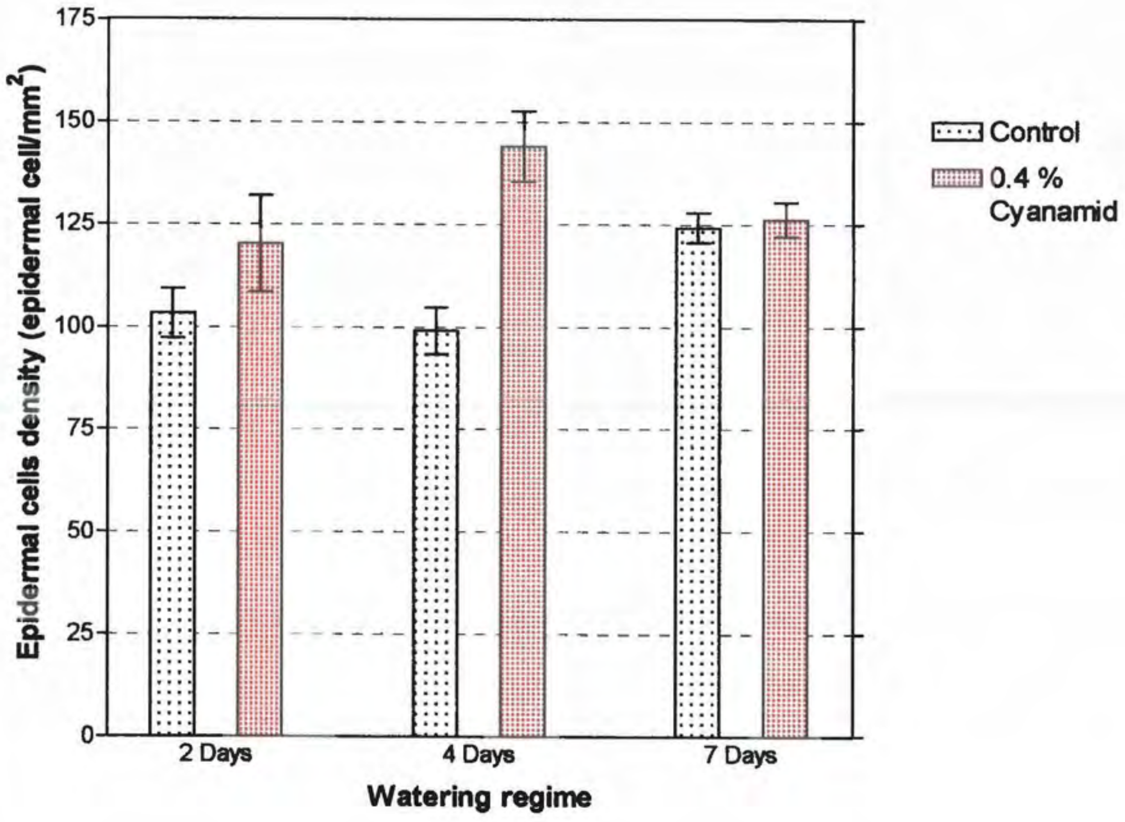


Figure 23

Stomatal Index measured in percent, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 23

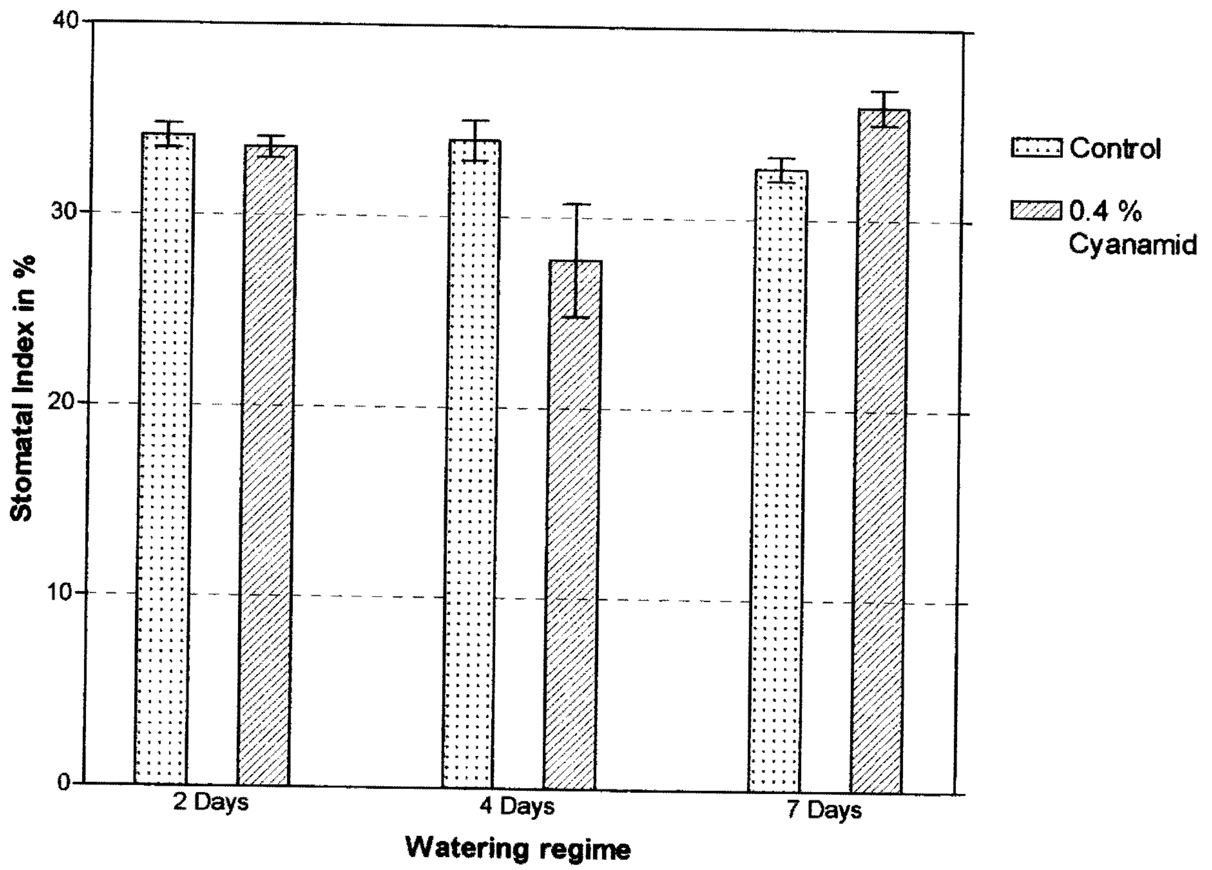
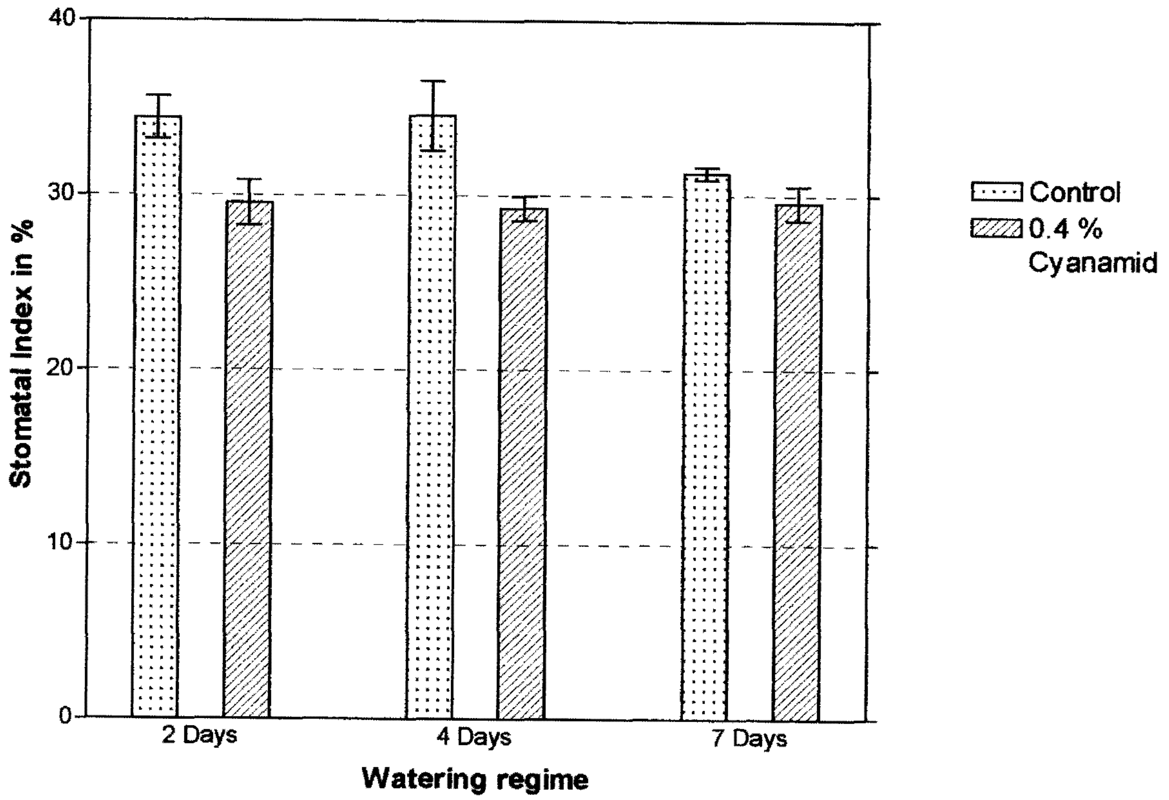


Figure 24

Stomatal Index measured in percent, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cyanamid.

Figure 24



3.1.10 The effect of commercial Cycocel

A similar lack of consistency of response was shown by wheat plants maintained under a 2-day regime following treatment of caryopses with Cycocel. Here stomatal pore length (Figure 25 & 26), Stomatal Density (Figure 27 & 28), epidermal cell size (Figure 29 & 30) and Stomatal Index (Figure 31 & 32) did not show a clear pattern of response which would be counted with a retention of water in plants.

Figure 25

Stomatal pore length in µm, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7-day watering regimes.

2 day watering = plants were watered every 2 days interval (continuous watering)

7 day watering = plant were watered every 7 days.

Figure 26

Stomatal pore length in µm, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7 day watering regimes.

2 day watering = plants were watered every 2 days interval (continuous watering)

7 day watering = plant were watered every 7 days.

Figure 25

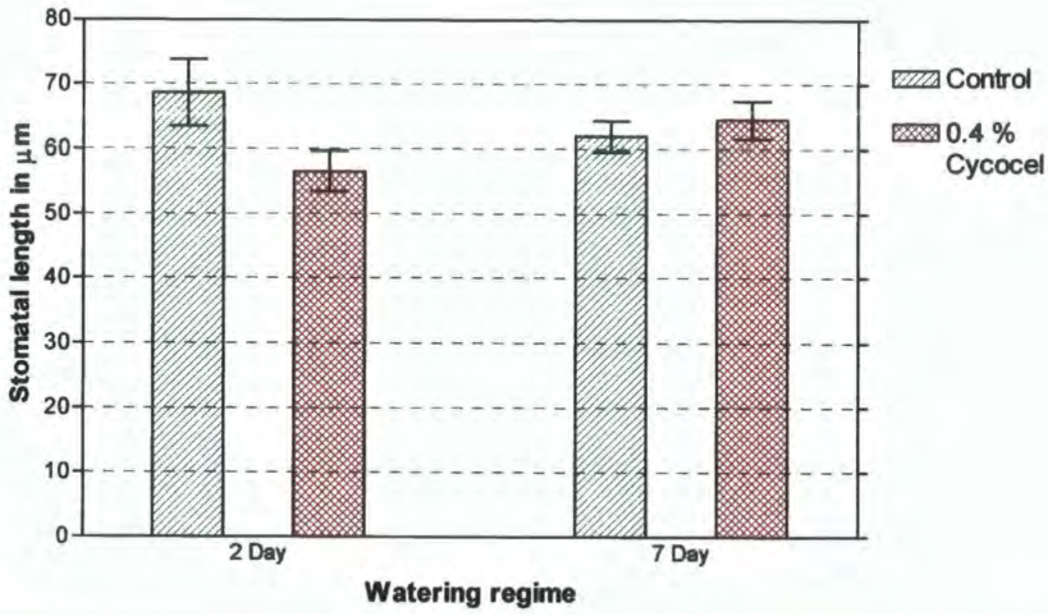


Figure 26

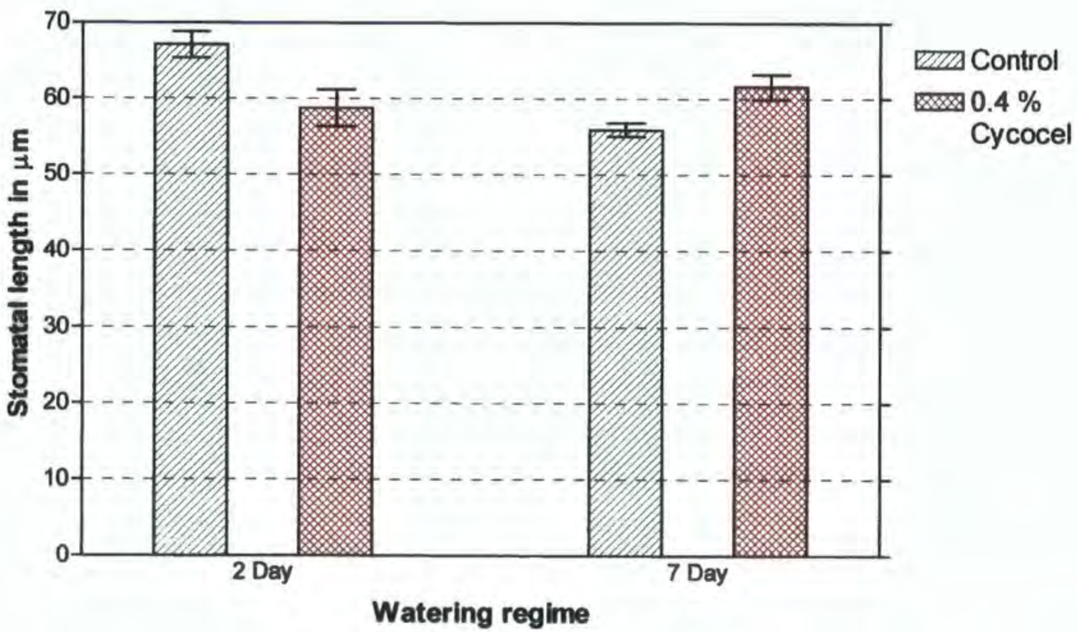


Figure 27

Stomatal Density measured as stomata/mm^2, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7-day watering regimes.

2 day watering = plants were watered every 2 days interval (continuous watering)

7 day watering = plant were watered every 7 days.

Figure 28

Stomatal Density measured as stomata/mm^2, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7 day watering regimes.

2 day watering = plants were watered every 2 days interval (continuous watering)

7 day watering = plant were watered every 7 days.

Figure 27

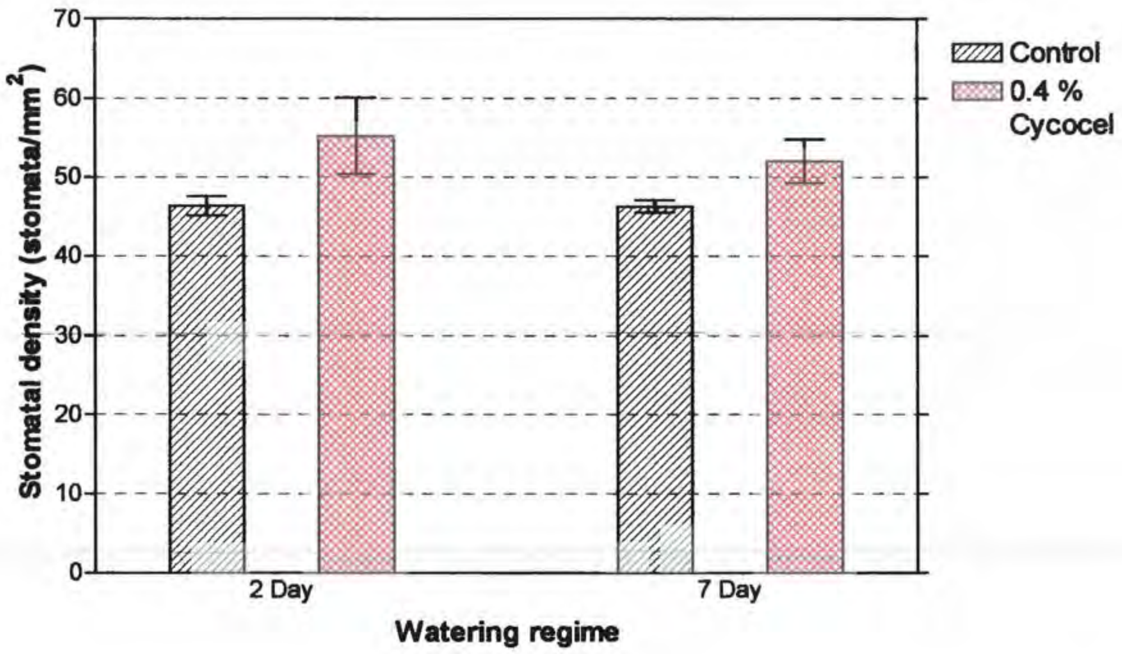


Figure 28

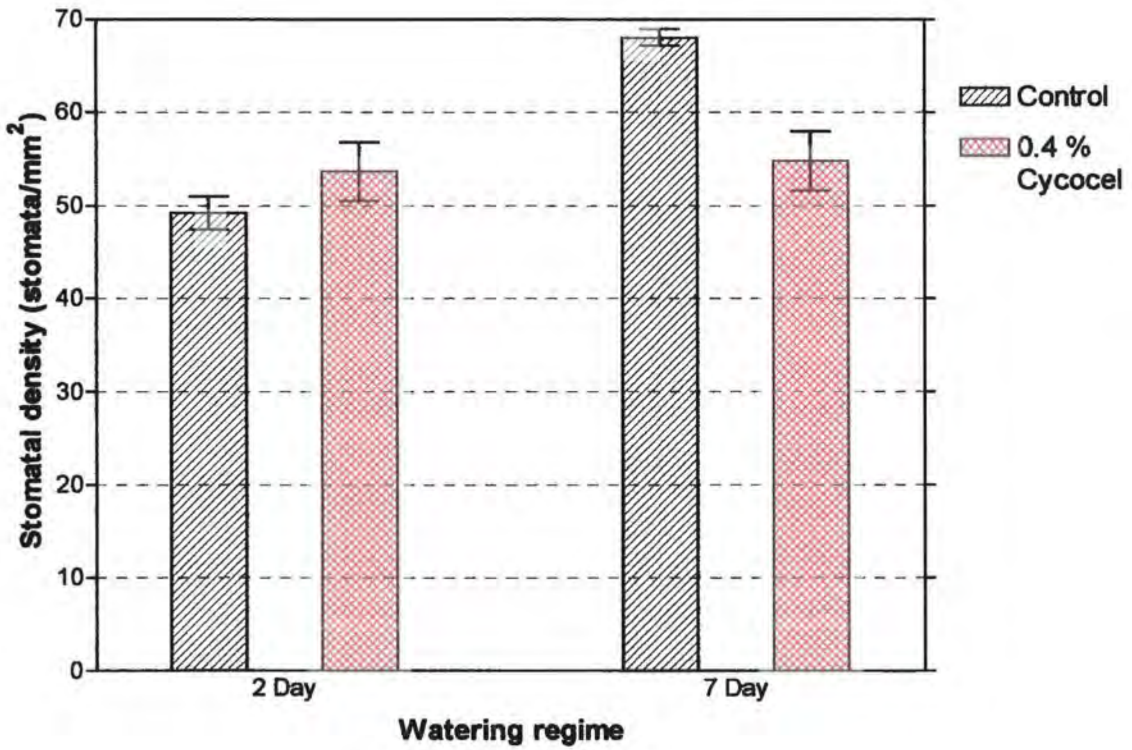


Figure 29

Epidermal cells density measured as epidermal cell/mm^2, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7-day watering regimes.

2 day watering = plants were watered every 2 days interval (continuous watering).

7 day watering = plant were watered every 7 days.

Figure 30

Stomatal Density measured as stomata/mm^2, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7 day watering regimes.

2 day watering = plants were watered every 2 days interval (continuous watering).

7 day watering = plant were watered every 7 days.

Figure 29

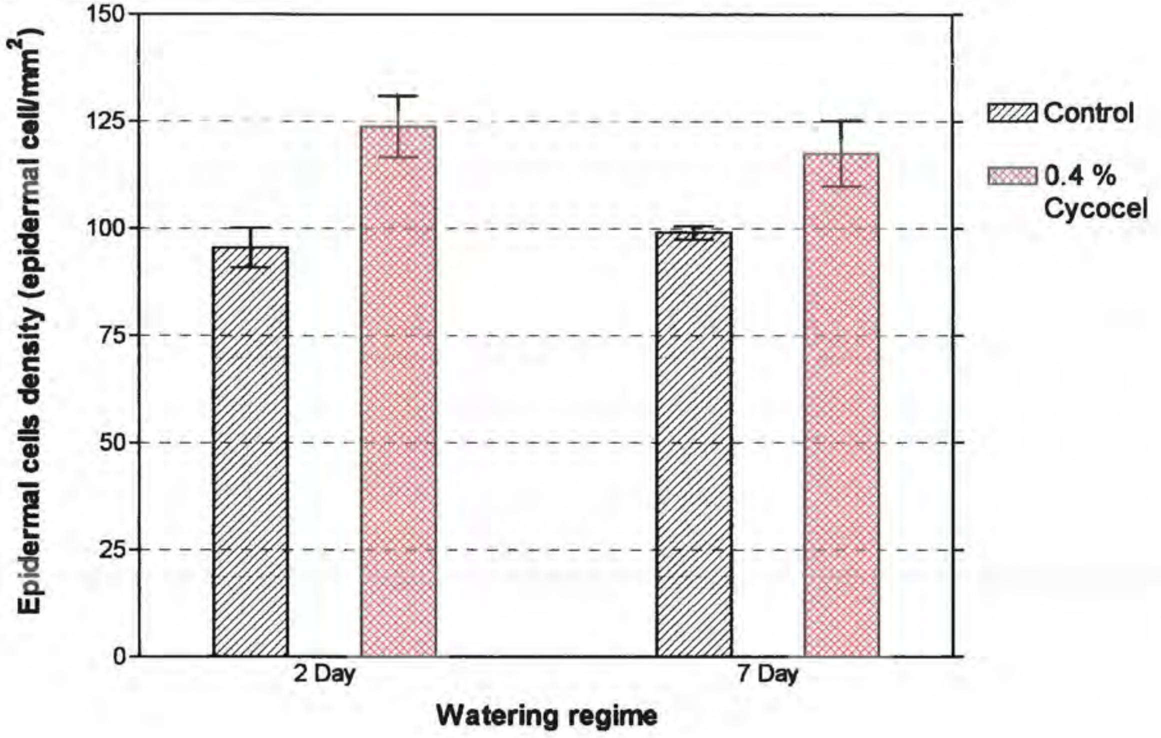


Figure 30

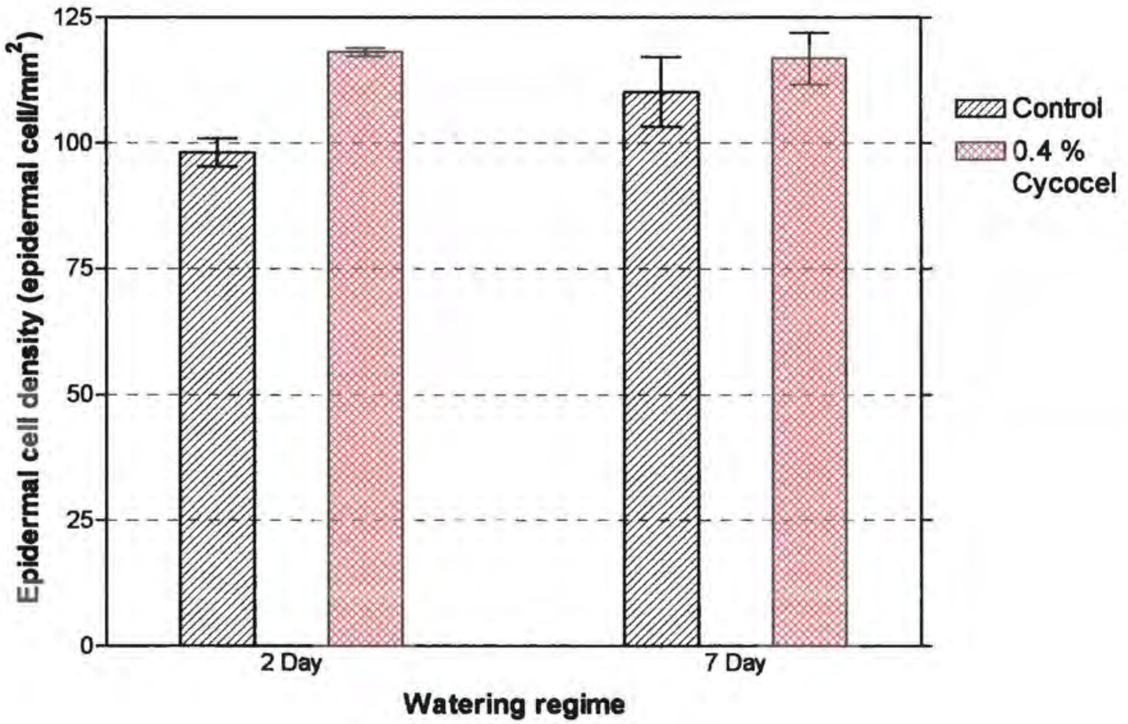


Figure 31

Stomatal Index measured in percent, as seen in the 3rd leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7-day watering regimes.

2 day watering = plants were watered every 2-3 days interval (continuous watering).

7 day watering = plant were watered every 7 days.

Figure 32

Stomatal Index measured in percent, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Seedlings were grown in 2 and 7 day watering regimes.

2 day watering = plants were watered every 2-3 days interval (continuous watering).

7 day watering = plant were watered every 7 days.

Figure 31

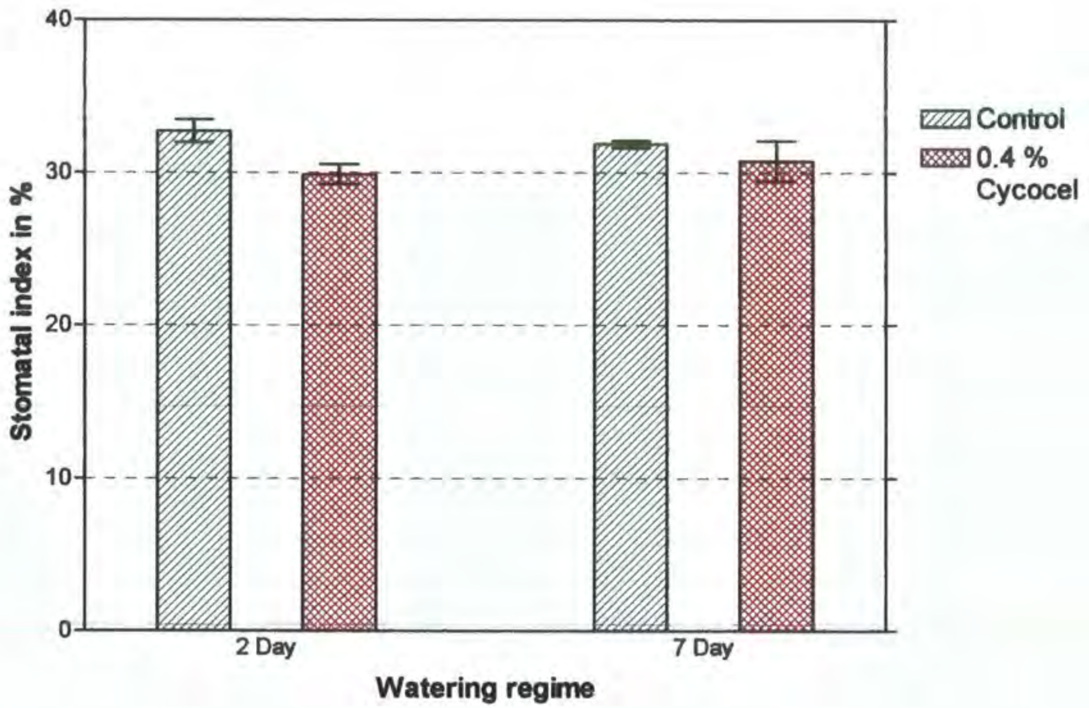
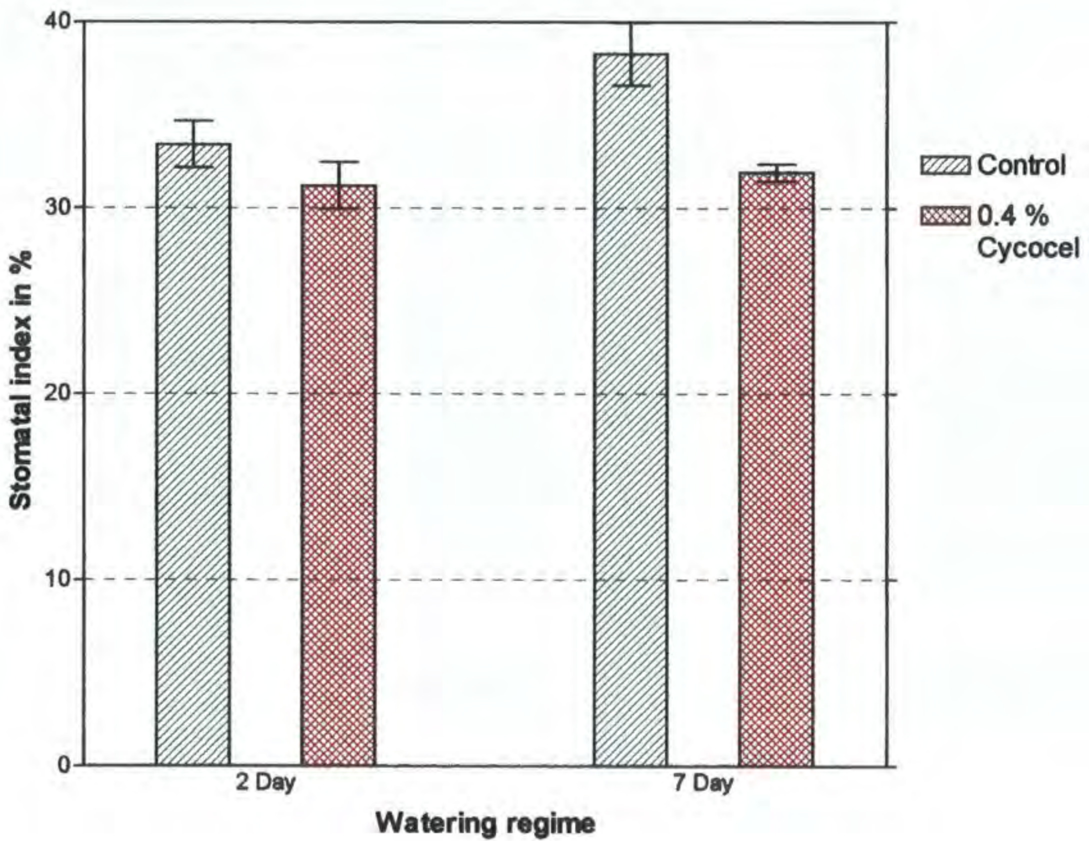


Figure 32



3.2 SECTION II: HYDROPONIC EXPERIMENTS

It was recognised that there were limitations involved in using pot experiments in order to manipulate water stress conditions even though there represented a more natural situations. In addition analyses of potential changes within the root systems were difficult because of the need to recover the tissue in an intact state and free of contamination. For these reasons further investigations of the influence of Cycocel on the water relations of wheat seedlings were performed using a honey jar hydroponic system coupled with Polyethylene Glycol (PEG) treatment. This allowed for a finer control of water-limitation treatment and access to roots.

3.2.1 Growth

Pre-germinated seedlings (caryopses germinated in 0.4 % Cycocel or water as before) were grown in honey jars filled with Nutrient Solution (NS) or the combination of Nutrient Solution and Polyethylene Glycol (NS + PEG), as described in the methods. The induced water stress state inhibited both apex and leaf elongation. As was seen for the pot experiments Cycocel pre-treatment stunted plant growth, but its effect was more obvious in the NS alone rather than in the NS + PEG trial.

3.2.2 Leaf length

The length of each leaf blade was measured when it reached maturity and stopped growing. As seen in Figure 33 the effect of PEG (at -5 bar) was to bring about a statistically significant ($P < 0.0001$) reduction in successive leaves of the wheat seedlings compared with those grown in NS alone. This was to be expected as an effect of water limitation.

Seedlings from caryopses pre-treated with 0.4 % Cycocel also showed reduced leaf blade length when grown in NS alone. However, the pattern of growth of successive leaves was different here from the control plants in that the early leaves were inhibited and the later leaves (3 & 4) showed better growth. However, when the treated seedling was grown in NS + PEG this effect was enhanced to the extent that the 3rd leaf showed equal growth to the comparable leaf in the control plants. The 4th leaf actually showed better growth than the control under the PEG treatment. It appears that Cycocel pre-treatment was countering the effect of PEG-induced water stress in terms of leaf growth.

3.2.3 Fresh and dry weight

The influence of Cycocel along with Cycocel + PEG on the fresh and dry weight of wheat seedlings were investigated. In this study wheat seedlings from water or Cycocel 0.4 % pre-treatment were grown in NS or in NS + PEG at -5 bar. The plants were harvested after 35 days of growth, fresh and dry weight were determined as in the methods for both shoots and roots. The data relating the fresh and dry weight are represented in Figure 34, 35, 36, and 37.

The conspicuous influence of Cycocel on leaf growth of the seedlings, was reflected in the fresh and dry weight analysis. For the leaf tissue whole plants were harvested so that the fresh and dry weight values represent an average for all the leaves. In the NS treatment alone, it was noted that Cycocel reduced both fresh and dry weight of the shoots compared with the control seedlings. This is counted with the results obtained for leaf length. Under PEG treated both the fresh and dry weight of the control plants was reduced. Again consistent with the effect of water stress

treatment. However, plants from Cycocel pre-treatments maintained the fresh and dry weight values compared to the NS alone. This indicates again that Cycocel is able to enhance growth as a counter to water stress treatment. There was no synergetic effect of water stress treatment and Cycocel.

Analysis of fresh and dry weight of root system from the same treatments were also made. Here the effect differed between the fresh and dry weight values. PEG treatment reduced the fresh weight of the root system of the control plants which is consistent with a reduction in cell size. However, dry weight of the roots was increased which reflects an increase in overall root tissue in terms of cells. This would be expected as a response to water stress where root to shoot ratio increases. Cycocel pre-treatment maintained the fresh weight of the roots and brought about significant increase over the control values (NS + PEG). This indicates that again Cycocel is enhancing the water holding component of tissues. No significant different was seen in the dry weight of these roots and the value was enhanced over the NS grown roots.

Figure 33

The influence of Cycocel on individual wheat leaf (blade) length, when grown hydroponically in honey jars.

NS = Hoagland nutrient solution

NS + PEG = Hoagland nutrient solution + Polyethylene Glycol at -5 bar

Control Caryopses were germinated in H_2O, whereas treated Caryopses were germinated in 0.4 % Cycocel.

Figure 33

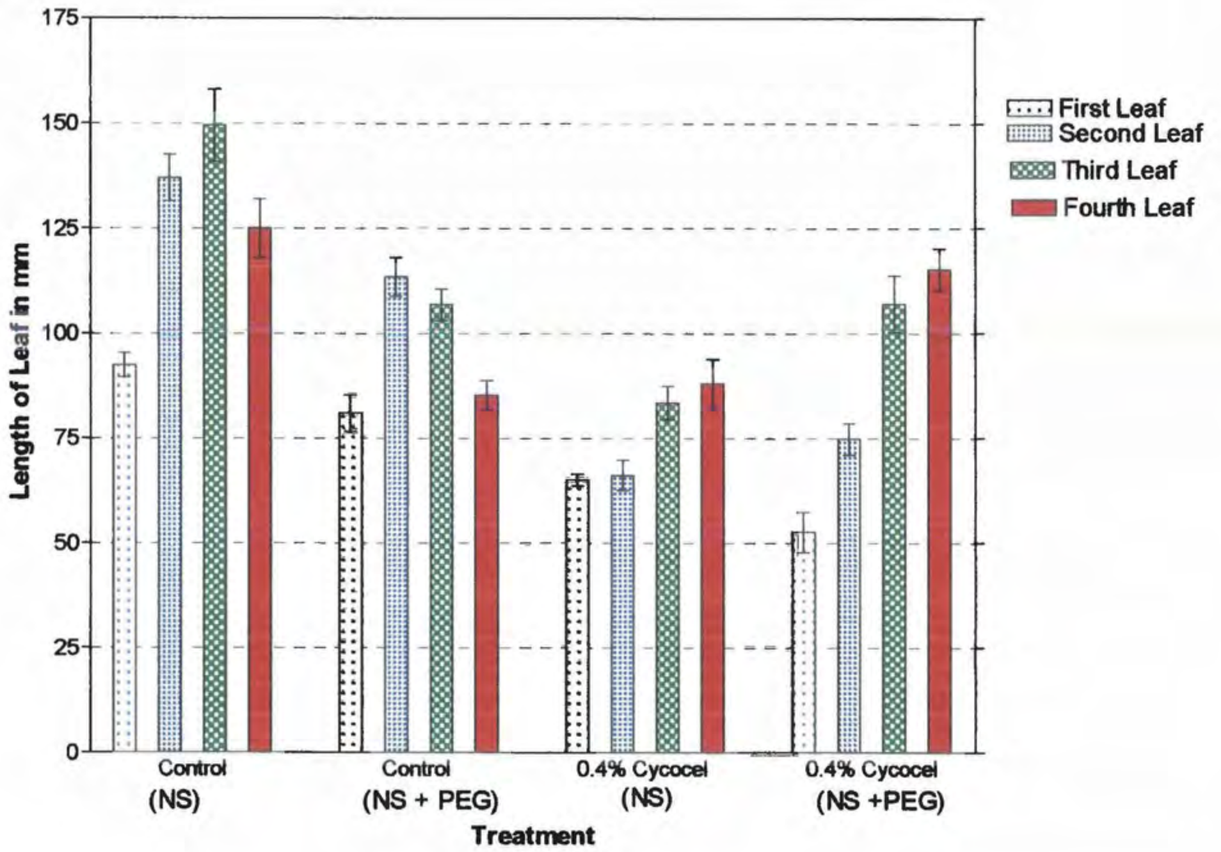


Figure 34

Fresh weight in gram of wheat shoots, when grown in NS and NS + PEG solutions.

NS = Hoagland nutrient solution

NS + PEG = Hoagland nutrient solution + Polyethylene Glycol at -5 bar.

Figure 35

Dry weight in gram of wheat shoots, when grown in NS and NS + PEG solutions.

NS = Hoagland nutrient solution.

NS + PEG = Hoagland nutrient solution + Polyethylene Glycol at -5 bar.

Figure 34

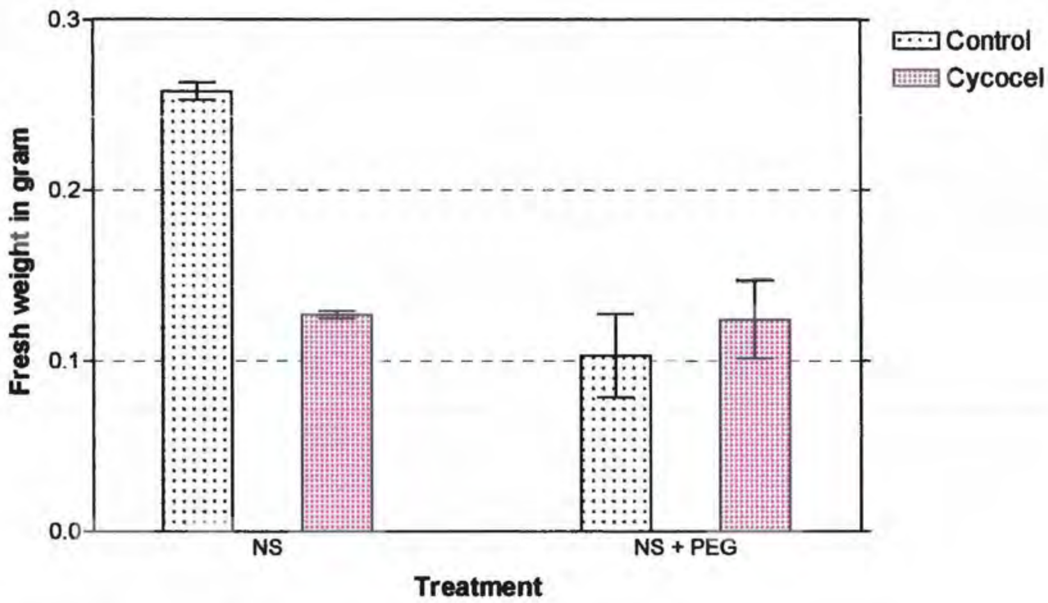


Figure 35

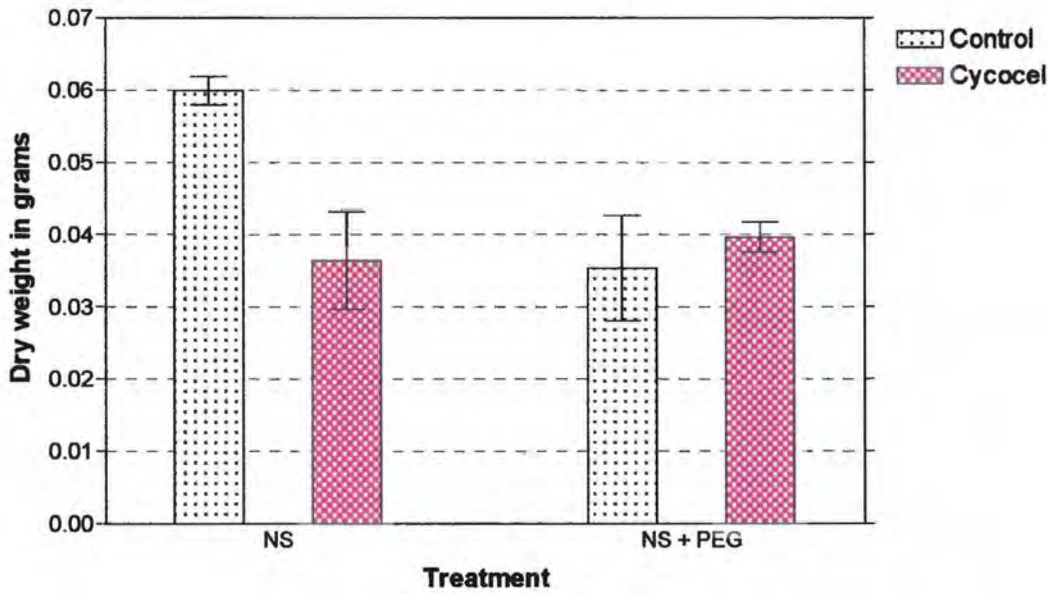


Figure 36

Fresh weight in gram of wheat roots, when grown in NS and NS + PEG solutions.

NS = Hoagland nutrient solution

NS + PEG = Hoagland nutrient solution + Polyethylene Glycol at -5 bar.

Figure 37

Dry weight in gram of wheat roots, when grown in NS and NS + PEG solutions.

NS = Hoagland nutrient solution

NS + PEG = Hoagland nutrient solution + Polyethylene Glycol at -5 bar.

Figure 36

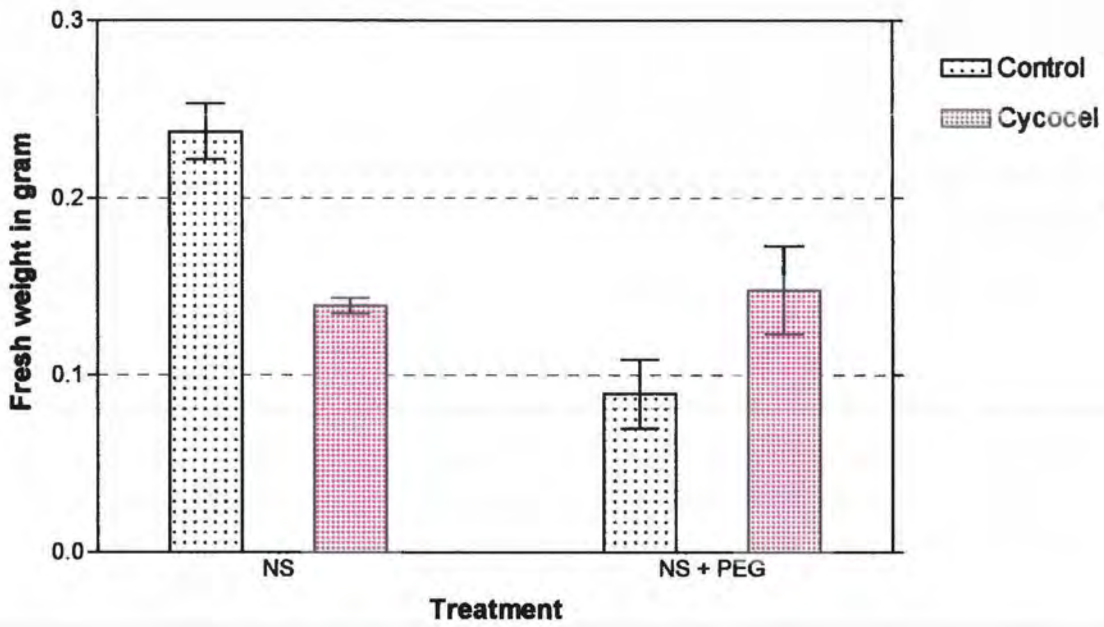
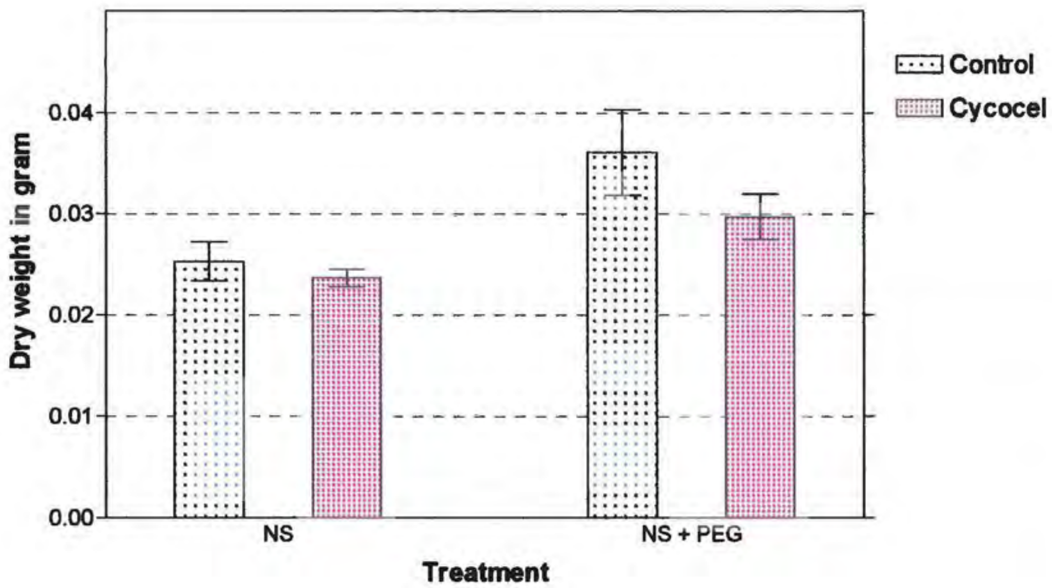


Figure 37



3.2.4 Proline Accumulation

The analysis of total amino acids extraction from wheat seedlings was performed to investigate any proline accumulation as a result of Cycocel pre-treatment. The analyses in this study included both shoots and roots, since hydroponic culture allowed this.

3.2.5 The influence of Cycocel on proline accumulation in shoots

As was seen in the pot experiments the plants grown in NS showed no proline accumulation when amino acids were analysed by TLC (Figure 38). Likewise Cycocel pre-treatment of caryopses did not result in the accumulation of proline in wheat seedlings in NS (Figure 39). However, again some variation in the R*f* values of the purple band was seen between different samples (Table 5).

Unlike in the pot experiments, when wheat seedlings were grown in NS + PEG at -5 bar, the total amino acids extraction from pre-treated seedlings did not show any accumulation of proline (Figure 40 &41). A similar result was seen for control tissues. R*f* values are given in Table 6. However, a yellow pigmentation spot at the approximate position of the proline standard was present prior to spraying with Ninhydrin and assumed therefore not to be an amino acid (Figure 40). No explanation is possible for the low R*f* of this fraction since it normally appeared at R*f* of 0.92. In the treated seedlings, an extra purple staining band appeared to be produced at R*f* 0.69 in response to the PEG (Figure 41). This, however, did not have the characteristic of proline. Proline appears not to have been produced in the shoots under any treatment in hydroponic culture.

Figure 38

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

NS = Hoagland Nutrient Solution.

Sample :-

30 - 50 μl of total amino acids from the plant shoots held in NS culture, derived from caryopses germinated water.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 38

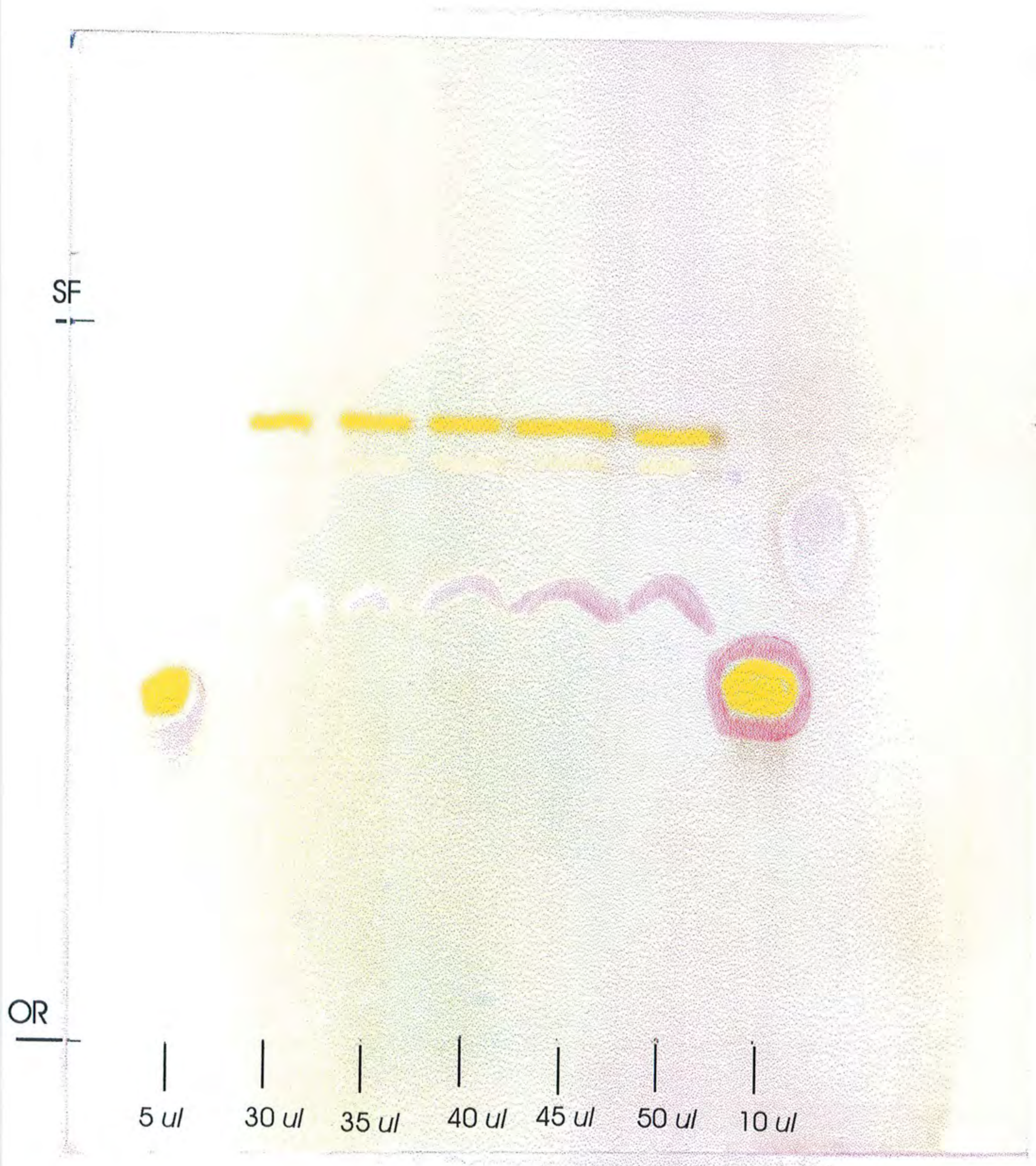


Figure 39

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

NS = Hoagland Nutrient Solution.

Sample :-

30 - 50 µl of total amino acids from the plant shoots held in NS culture, derived from caryopses germinated 0.4 % Cycocel.

Marker:-

5 and 10 µl of standard L-proline solution (outside tracks).

Figure 39

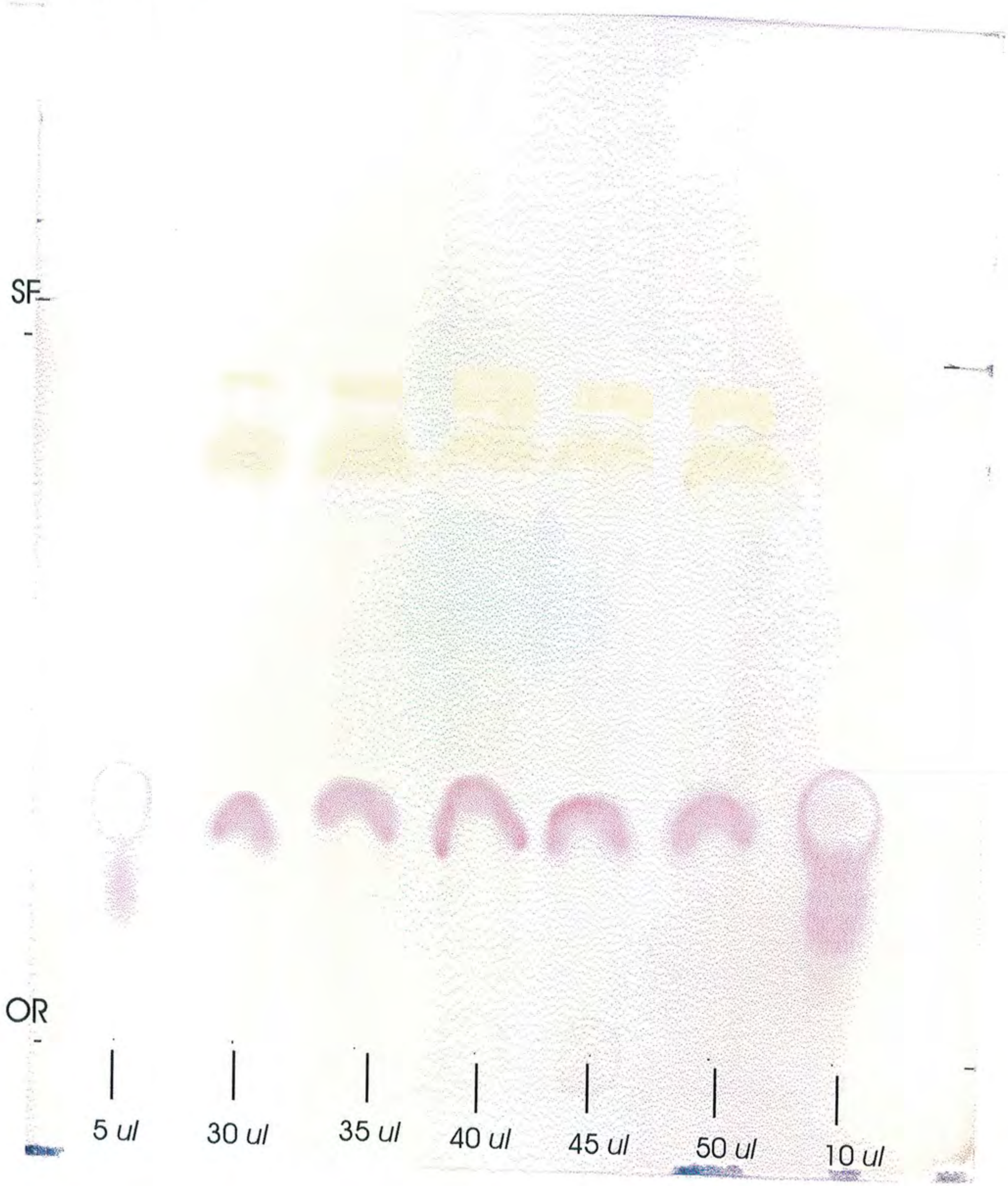


Table 5.

The Rf - values of proline and amino acids bands extracted from wheat leaf tissue.

Control:-

plants from wheat caryopses germinated in water.

Treated:-

plants from wheat caryopses germinated in 0.4 % Cycocel.

Visible pigments was seen before spraying with Ninhydrin.

Table 5

Treatment	Growth media	Concentration *ul* [1]	Proline	Colour of spot	Unknown band	Colour of spot	Unknown band	Colour of spot	visible Pigment	Colour of spot	visible Pigment	Colour of spot
Control			*Rf*		*Rf*		*Rf*		*Rf*		*Rf*	
	NS *	*5 ul*	0.55	Yellow								
		30 ul			0.71	Light Pink			0.91	Yellow	0.99	Yellow
		35 ul			0.72	Light Pink			0.91	Yellow	0.99	Yellow
		40 ul			0.74	Light Pink			0.93	Yellow	0.99	Yellow
		45 ul			0.73	Light Pink			0.93	Yellow	0.99	Yellow
		50 ul			0.74	Light Pink			0.91	Yellow	0.99	Yellow
		10 ul	0.58	Yellow								
Treated												
	NS *	*5 ul*	0.36	Yellow								
		30 ul			0.35	Light Pink			0.92	Yellow	0.97	Yellow
		35 ul			0.37	Light Pink			0.92	Yellow	0.96	Yellow
		40 ul			0.37	Light Pink			0.93	Yellow	0.94	Yellow
		45 ul			0.35	Light Pink			0.93	Yellow	0.94	Yellow
		50 ul			0.36	Light Pink			0.93	Yellow	0.94	Yellow
		10 ul	0.45	Yellow								

* Plants were grown in Hoagland Nutrient Solution.

Figure 40

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

NS + PEG = Hoagland Nutrient Solution and Polyethylene Glycol.

Sample :-

30 - 50 μl of total amino acids from the plant shoots held in NS + PEG culture, derived from caryopses germinated in water.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 40

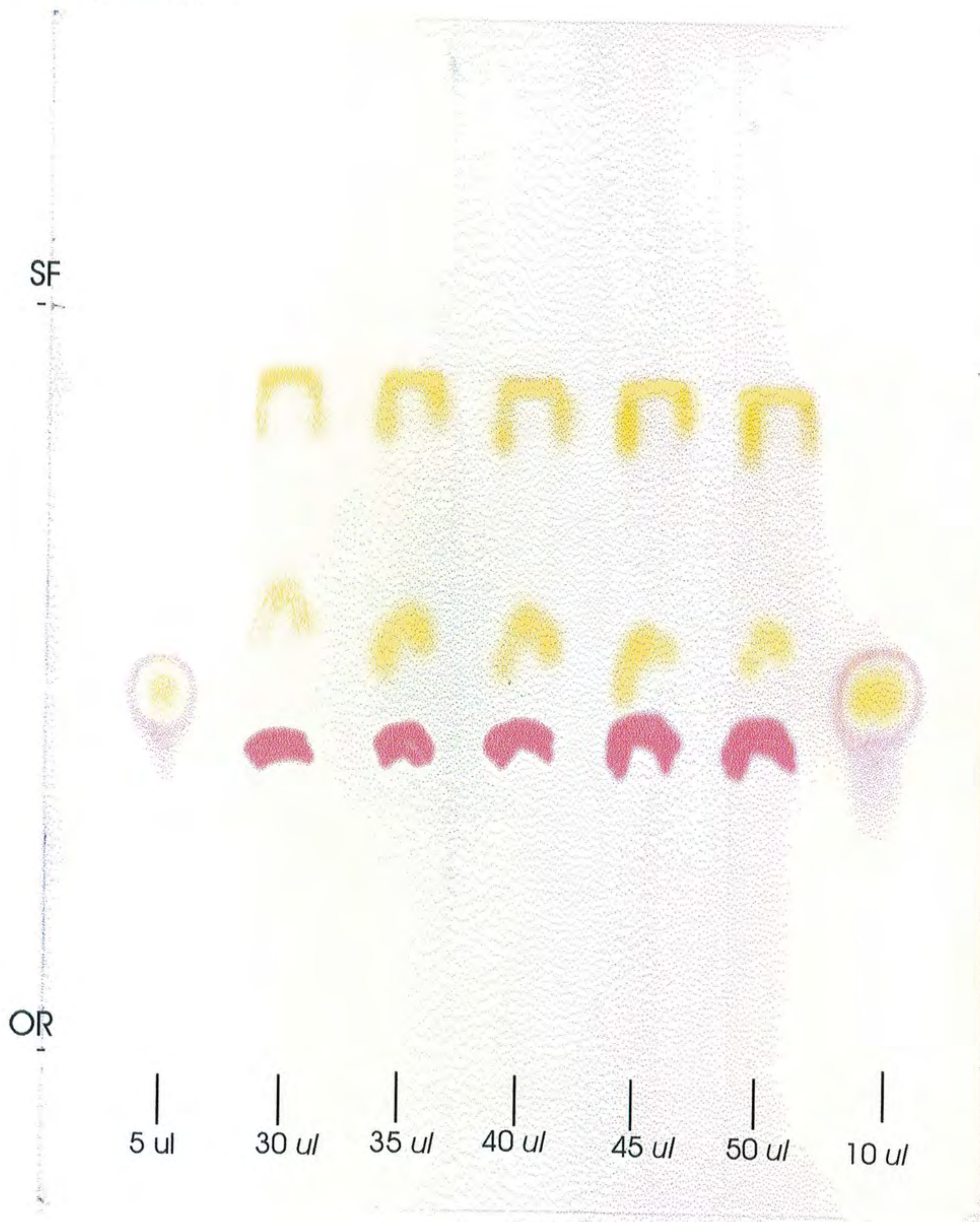


Figure 41.

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat leaves.

SF = solvent front.

OR = origin of loading.

NS + PEG = Hoagland Nutrient Solution and Polyethylene Glycol.

Sample :-

30 - 50 μl of total amino acids from the plant shoots held in NS + PEG culture, derived from caryopses germinated in 0.4 % Cycocel.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 41

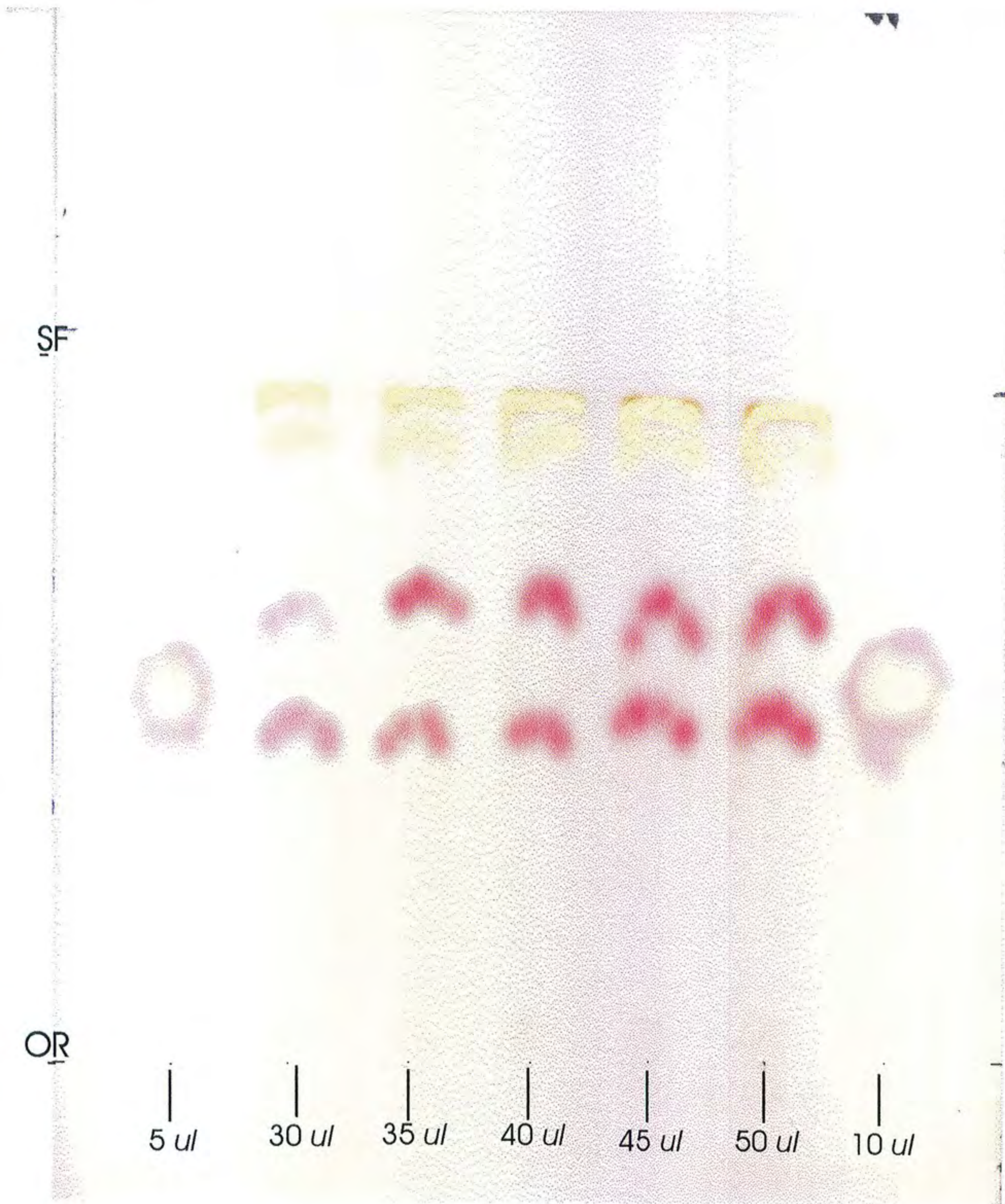


Table 6.

The R_f - values of proline and amino acids bands extracted from wheat leaf tissue.

Control:-

plants from wheat caryopses germinated in water.

Treated:-

plants from wheat caryopses germinated in 0.4 % Cycocel.

Visible pigments was seen before spraying with Ninhydrin.

Table 6

Treatment	Growth media	Concentration (*ul*)	Proline	Colour of spot	Unknown band	Colour of spot	Unknown band	Colour of spot	visible Pigment	Colour of spot	visible Pigment	Colour of spot
Control			R*f*		R*f*		R*f*		R*f*		R*f*	
	NS + PEG *	*5 ul*	0.52	Yellow								
		30 ul			0.45	Light Pink			0.68	Yellow	0.97	Yellow
		35 ul			0.45	Light Pink			0.64	Yellow	0.97	Yellow
		40 ul			0.46	Light Pink			0.64	Yellow	0.97	Yellow
		45 ul			0.47	Light Pink			0.62	Yellow	0.97	Yellow
		50 ul			0.47	Light Pink			0.65	Yellow	0.97	Yellow
		10 ul	0.54	Yellow								
Treated												
	NS + PEG *	*5 ul*	0.55	Yellow								
		30 ul			0.50	Light Pink	0.68	Light Pink	0.92	Yellow	0.99	Yellow
		35 ul			0.50	Light Pink	0.70	Light Pink	0.93	Yellow	0.99	Yellow
		40 ul			0.50	Light Pink	0.69	Light Pink	0.92	Yellow	0.99	Yellow
		45 ul			0.52	Light Pink	0.68	Light Pink	0.92	Yellow	0.99	Yellow
		50 ul			0.52	Light Pink	0.69	Light Pink	0.91	Yellow	0.99	Yellow
		10 ul	0.57	Yellow								

* Plants were grown in Hoagland Nutrient Solution + Polyethylene glycol (NS + PEG) at -5 bar.

3.2.6 The influence of Cycocel on proline accumulation in roots

Analysis of proline accumulation in the plant roots was also undertaken under both NS and NS + PEG solutions with control and Cycocel pre-treated plants.

Under NS solution, the roots of neither the control nor the Cycocel-treated plants appear to have no indication of any proline accumulation (Figure 42 & 43). There was, however, similar pattern of bands in both treated and untreated (Figure 42 & 43), but the Rf-values of the unknown bands in the Cycocel-treated roots were lower than their counterpart of the control (Table 7). No extracted total proline accumulation was noted under NS + PEG solution for either the control and treated plants. Interestingly, the amino acids in both treated and control when grown under NS + PEG produced similar patterns of bands consistently (Figure 44 & 45). However, again there was variation in the absolute Rf value for many of the bands (Table 8). This may have been due to high salt availability to the plants under these growth conditions.

Given the greater variation in the analysed samples of plant from hydroponic culture it was not possible to derive absolute conclusions about the effect of Cycocel on proline accumulation.

Figure 42

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat roots.

SF = solvent front.

OR = origin of loading.

NS = Hoagland Nutrient Solution.

Sample :-

30 - 50 µl of total amino acids from the plant roots held in NS culture, derived from caryopses germinated in 0.4 % Cycocel.

Marker:-

5 and 10 µl of standard L-proline solution (outside tracks).

Figure 42

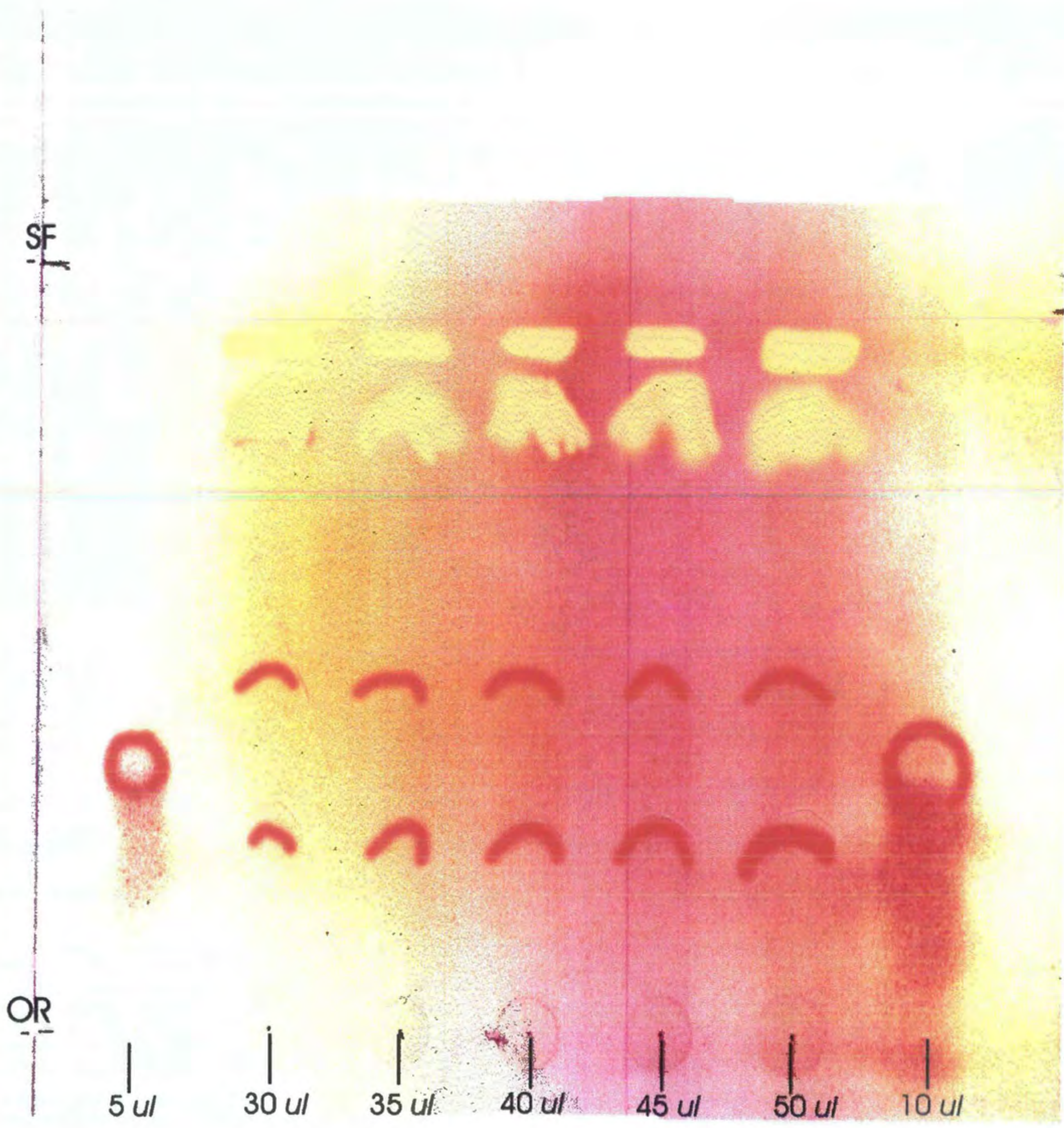


Figure 43

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat roots.

SF = solvent front.

OR = origin of loading.

NS = Hoagland Nutrient Solution.

Sample :-

30 - 50 μl of total amino acids from the plant roots held in NS culture, derived from caryopses germinated in water.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 43

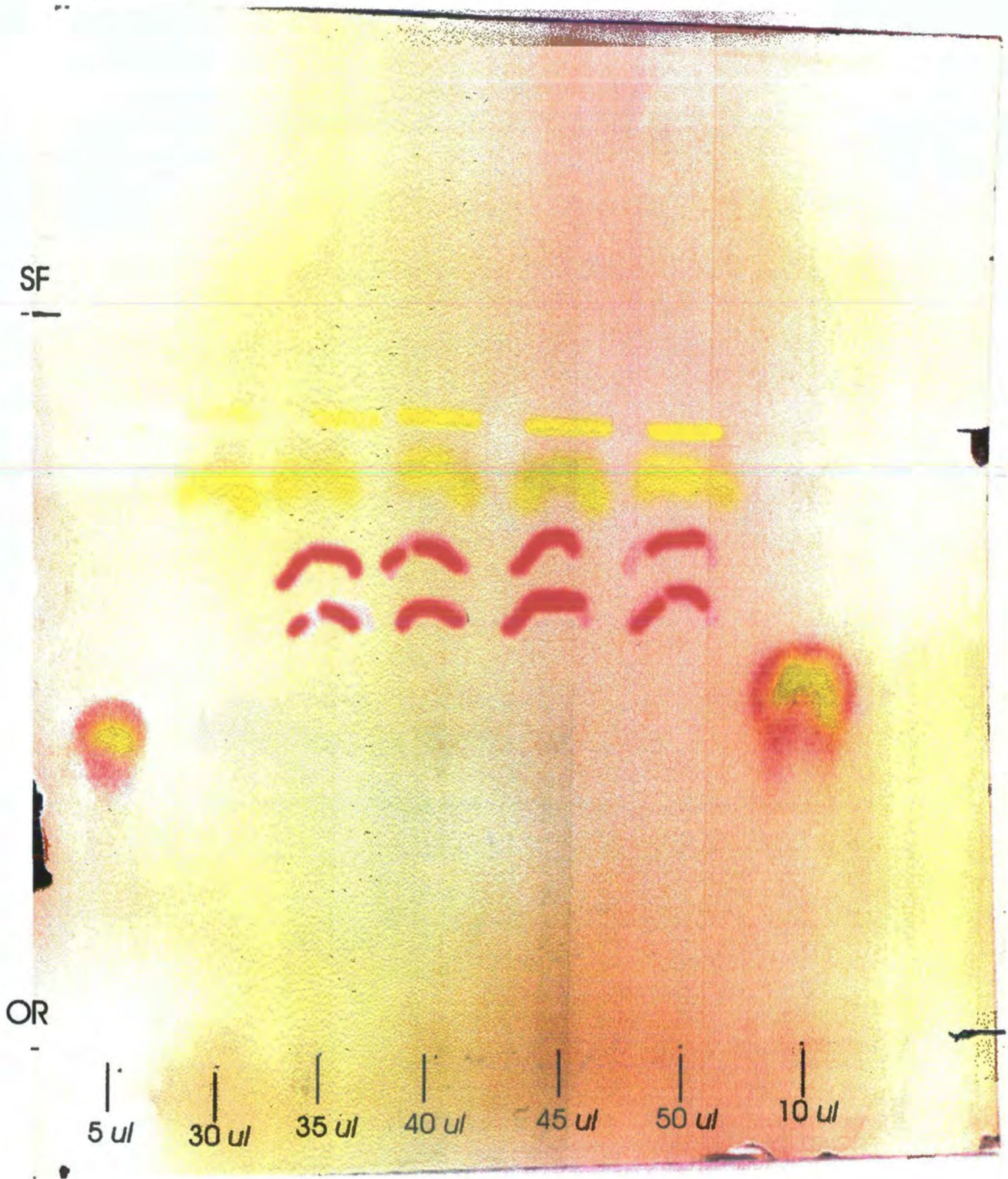


Table 7.

The Rf - values of proline and amino acids bands extracted from wheat root tissue.

Control:-

plants from wheat caryopses germinated in water.

Treated:-

plants from wheat caryopses germinated in 0.4 % Cycocel.

Visible pigments was seen before spraying with Ninhydrin.

Table 7

Treatment	Growth media	Concentration (*ul*)	Proline	Colour of spot	Unknown band	Colour of spot	Unknown band	Colour of spot	visible Pigment	Colour of spot	visible Pigment	Colour of spot
Control			*Rf*		*Rf*		*Rf*		*Rf*		*Rf*	
	N.S *	*5 ul*	0.4	Yellow								
		30 ul			0.60	Light Pink			0.82	Yellow	0.88	Yellow
		35 ul			0.60	Light Pink	0.71	Light Pink	0.80	Yellow	0.88	Yellow
		40 ul			0.63	Light Pink	0.73	Light Pink	0.81	Yellow	0.88	Yellow
		45 ul			0.65	Light Pink	0.74	Light Pink	0.80	Yellow	0.87	Yellow
		50 ul			0.67	Light Pink	0.72	Light Pink	0.82	Yellow	0.87	Yellow
		10 ul	0.48	Yellow								
Treated												
	N.S *	*5 ul*	0.37	Yellow								
		30 ul		Yellow	0.31	Light Pink	0.49	Light Pink	0.91	Yellow	0.94	Yellow
		35 ul		Yellow	0.30	Light Pink	0.49	Light Pink	0.93	Yellow	0.94	Yellow
		40 ul		Yellow	0.30	Light Pink	0.50	Light Pink	0.91	Yellow	0.94	Yellow
		45 ul		Yellow	0.30	Light Pink	0.49	Light Pink	0.92	Yellow	0.94	Yellow
		50 ul		Yellow	0.30	Light Pink	0.48	Light Pink	0.91	Yellow	0.94	Yellow
		10 ul	0.38	Yellow								

* Plants were grown in Hoagland solution (NS alone).

Figure 44

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat roots.

SF = solvent front.

OR = origin of loading.

NS + PEG = Hoagland Nutrient Solution and Polyethylene Glycol.

Sample :-

30 - 50 μl of total amino acids from the plant roots held in NS + PEG culture, derived from caryopses germinated in 0.4 % Cycocel.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 44

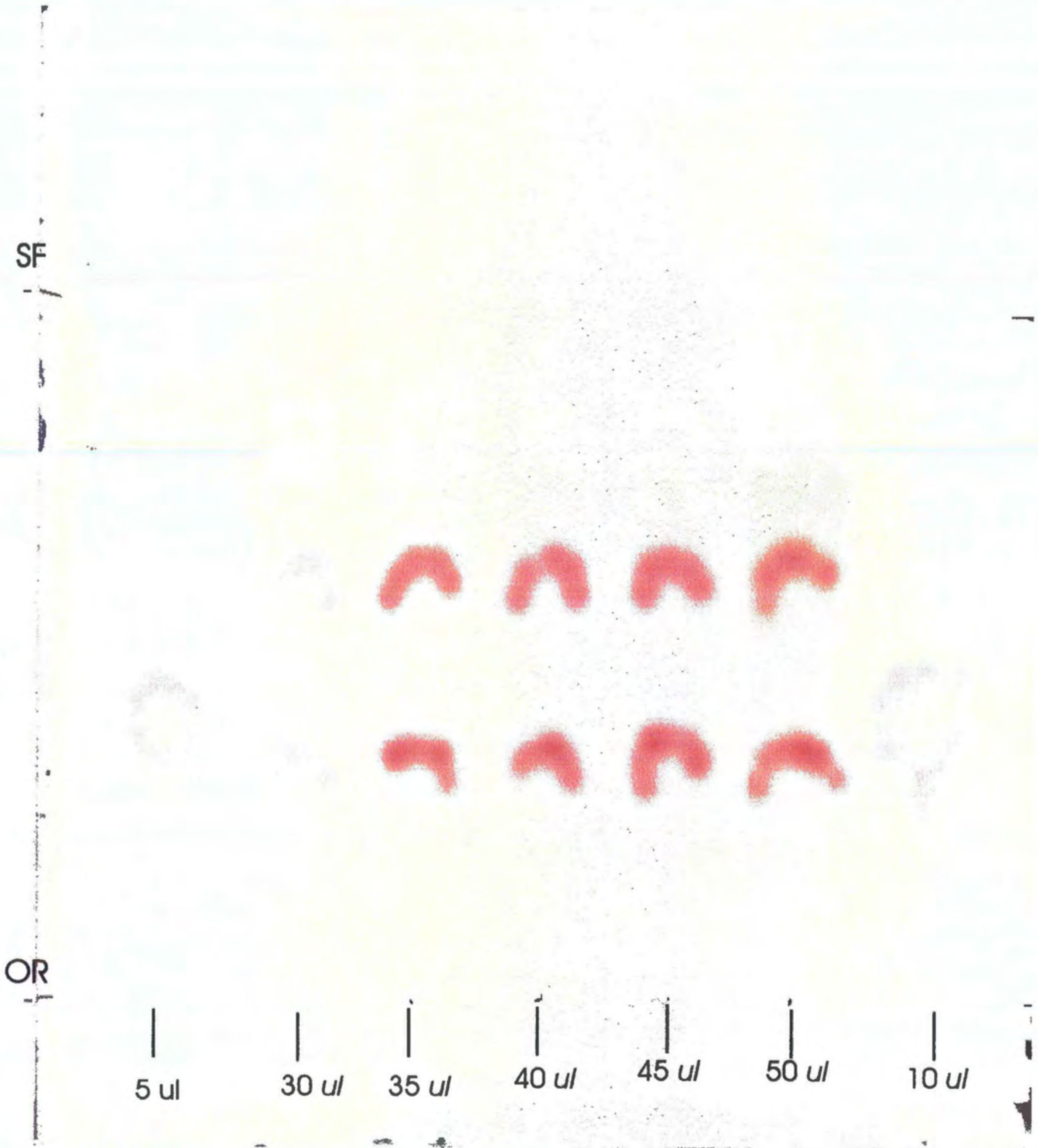


Figure 45

Enhanced colour scan of a Ninhydrin-treated TLC plate for total amino acids from wheat roots.

SF = solvent front.

OR = origin of loading.

NS + PEG = Hoagland Nutrient Solution and Polyethylene Glycol.

Sample :-

30 - 50 μl of total amino acids from the plant roots held in NS + PEG culture, derived from caryopses germinated in water.

Marker:-

5 and 10 μl of standard L-proline solution (outside tracks).

Figure 45

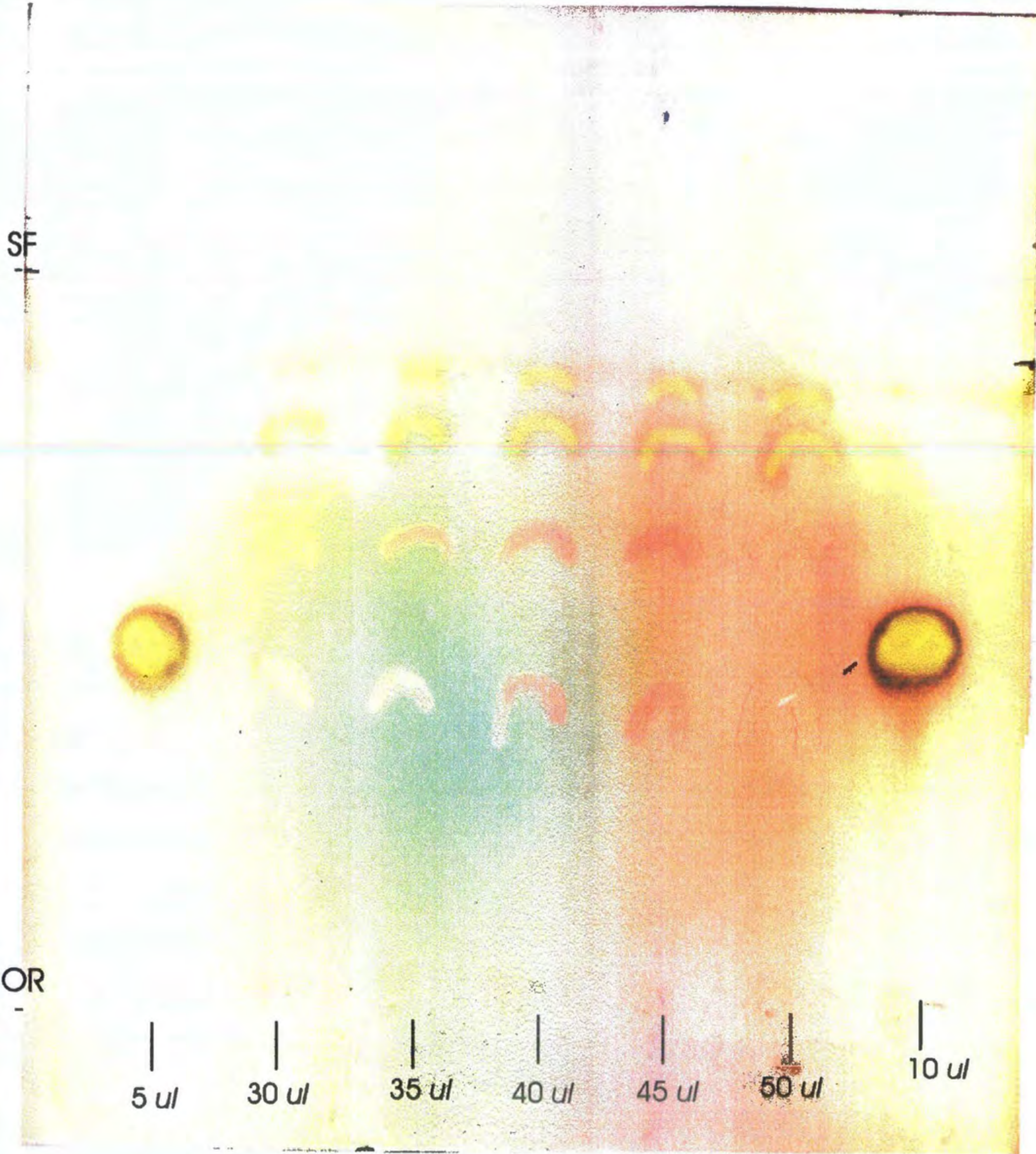


Table 8.

The Rf - values of proline and amino acids bands extracted from wheat root tissue.

Control:-

plants from wheat caryopses germinated in water.

Treated:-

plants from wheat caryopses germinated in 0.4 % Cycocel.

Visible pigments was seen before spraying with Ninhydrin.

Table 8

Treatment	Growth media	Concentration (*ul*)	Proline	Colour of spot	Unknown band	Colour of spot	Unknown band	Colour of spot	visible Pigment	Colour of spot	visible Pigment	Colour of spot
Control			Rf		Rf		Rf		Rf		Rf	
	NS + PEG *	*5 ul*	0.54	Yellow								
		30 ul			0.51	Light Pink	0.71	Light Pink	0.89	Yellow	0.96	Yellow
		35 ul			0.50	Light Pink	0.71	Light Pink	0.88	Yellow	0.96	Yellow
		40 ul			0.50	Light Pink	0.73	Light Pink	0.89	Yellow	0.96	Yellow
		45 ul			0.50	Light Pink	0.72	Light Pink	0.89	Yellow	0.96	Yellow
		50 ul			0.50	Light Pink	0.73	Light Pink	0.88	Yellow	0.96	Yellow
		10 ul	0.58	Yellow								
Treated												
	NS + PEG *	*5 ul*	0.41	Yellow								
		30 ul		Yellow	0.36	Light Pink	0.65	Light Pink	0.78	Yellow	0.87	Yellow
		35 ul		Yellow	0.37	Light Pink	0.64	Light Pink	0.78	Yellow	0.87	Yellow
		40 ul		Yellow	0.37	Light Pink	0.64	Light Pink	0.78	Yellow	0.87	Yellow
		45 ul		Yellow	0.38	Light Pink	0.64	Light Pink	0.78	Yellow	0.87	Yellow
		50 ul		Yellow	0.37	Light Pink	0.64	Light Pink	0.78	Yellow	0.87	Yellow
		10 ul	0.44	Yellow								

* Plants were grown in Hoagland Nutrient Solution + Polyethylene glycol (NS + PEG) at -5 bar.

3.2.7 Stomata

Earlier work with pot-grown plants had indicated an inconsistency in the stomatal characteristics found in response to Cycocel pre-treatment. This further investigation was initiated to try to underpin the relationship between Cycocel pre-treatment and stomatal characteristics using more refined conditions of hydroponic culture. The influence of Cycocel pre-treatment, at 0.4 %, of wheat caryopses on the length of stomatal pore, Stomatal Density and index, and the measurement of epidermal cells density was examined for plants grown in NS and NS + PEG conditions, as described earlier.

Under NS alone, it was observed that Cycocel treatment of wheat caryopses did reduce the length of the stomatal pores present in the seedling 4th leaf (Figure 46). This effect may be attributed to the overall stunting ability of Cycocel, but not to the influence of limited water availability. However, this particular relationship (reduced stomatal pore size) was not maintained when the pre-treated seedlings were grown in NS + PEG at -5 bar (Figure 46). Cycocel pre-treatment in combination with PEG-treatment of seedlings altered the length of stomatal pore in relation to NS growth conditions alone. However, no significant difference was found between the control and treated plants grown in PEG.

It was also noticed that Cycocel pre-treatment of caryopses resulted in a marked Stomatal Density increase. As seen from Figure 47, when wheat seedlings were grown in NS and NS + PEG at -5 bar, the Stomatal Density was found to be significantly higher ($P < 0.0001$). Cycocel pre-treatment did increase the density of stomata over the control in both trials, but this was not linked to a reciprocal decrease in pore size.

Figure 48 indicates that the overall response of epidermal cell density was found not to be statistically significant with either Cycocel or PEG treatment.

Therefore epidermal cell numbers (per given area) were not altered by the Cycocel treatment. As was seen for the pot experiments the changes in density of stomata were the results of increases in these cells as opposed to a decrease in epidermal cell size. From Figure 49, it appears that there was a general increase in the Stomatal Index of 4th leaves of seedlings from pre-treated caryopses. Cycocel treatment resulted in a significant increase ($P < 0.0001$) in Stomatal Index which was sampled from both trials. No significant difference was found in the Stomatal Index for tissue from NS or NS + PEG so an enhanced response to water limitation was not seen for this parameter. This stomatal proportion increase as a result of Cycocel pre-treatment is the opposite of what may have been expected for a reduction in water loss.

The increase in Stomatal Index in response to Cycocel pre-treated also indicates that this compound can influence the number of stomata which may be initiated for epidermal cells of the leaf. Cell differentiation appears to be influenced by Cycocel.

Figure 46

Stomatal pore length in µm, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Then seedlings were grown in NS and NS + PEG culture solution.

NS = Hoagland Nutrient Solution.

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol.

Figure 46

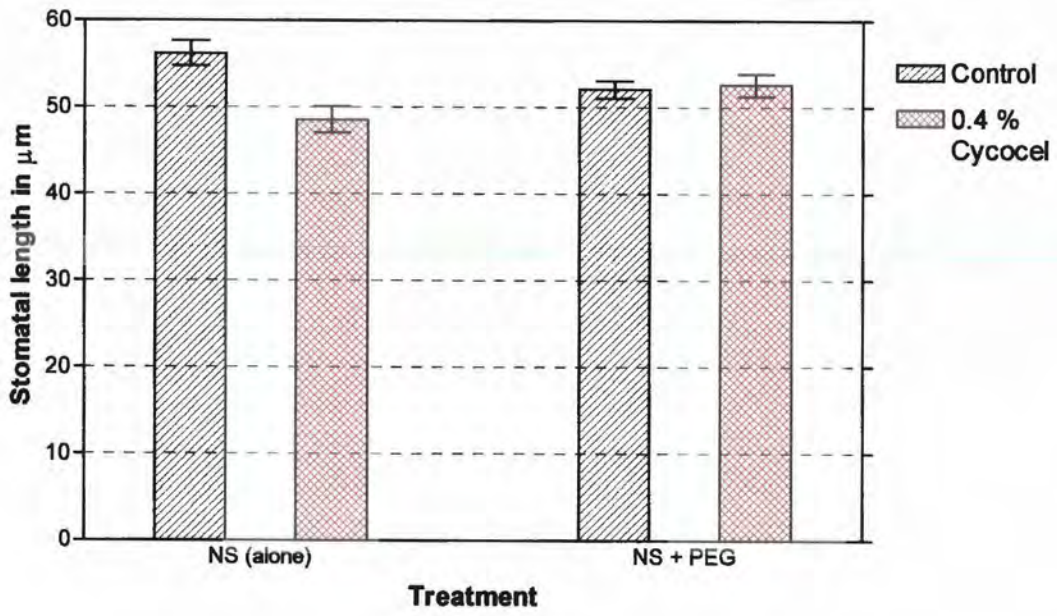


Figure 47

Stomatal Density measured as stomata/mm^2, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Then seedlings were grown in NS and NS + PEG culture solution.

NS = Hoagland Nutrient Solution.

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol.

Figure 47

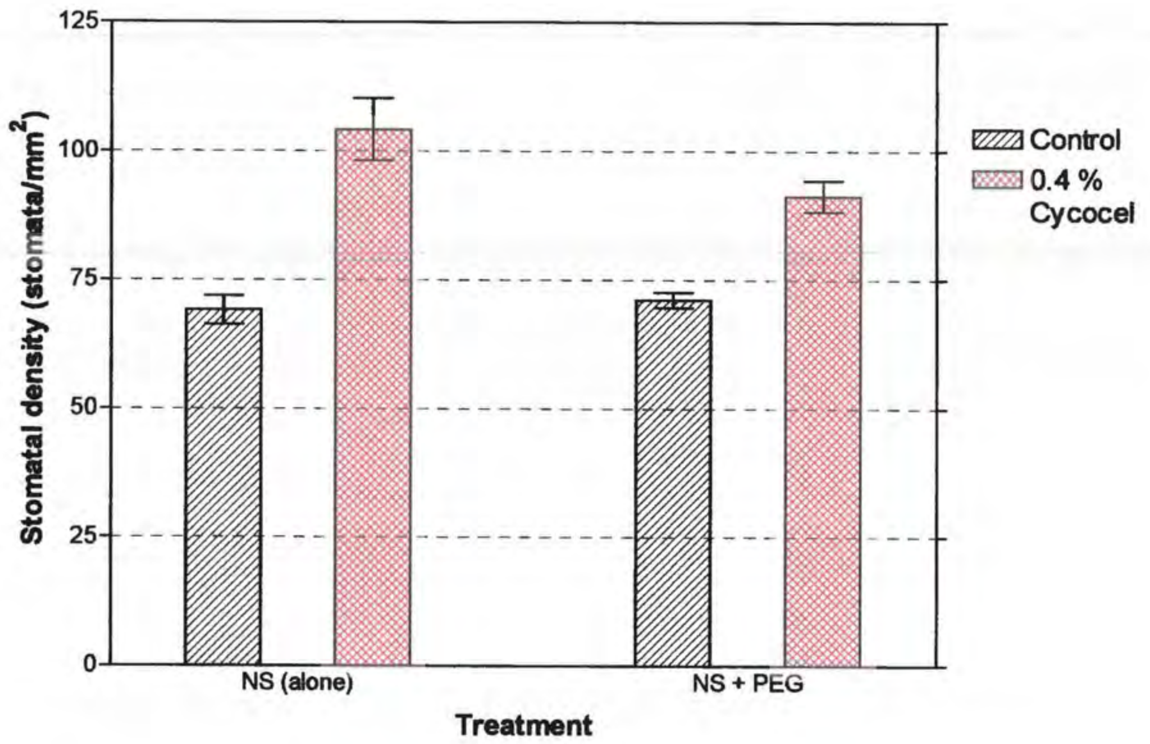


Figure 48

Epidermal cells density measured as epidermal cell/mm^2, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Then seedlings were grown in NS and NS + PEG solution.

NS = Hoagland Nutrient Solution.

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol.

Figure 48

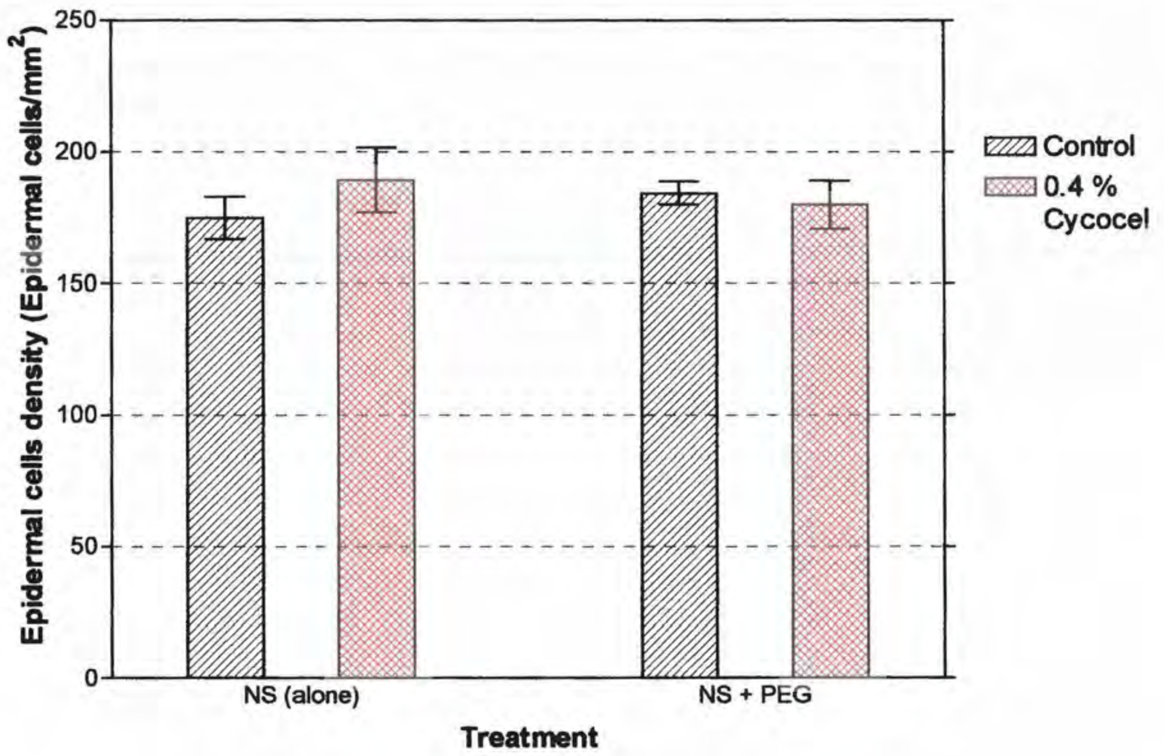


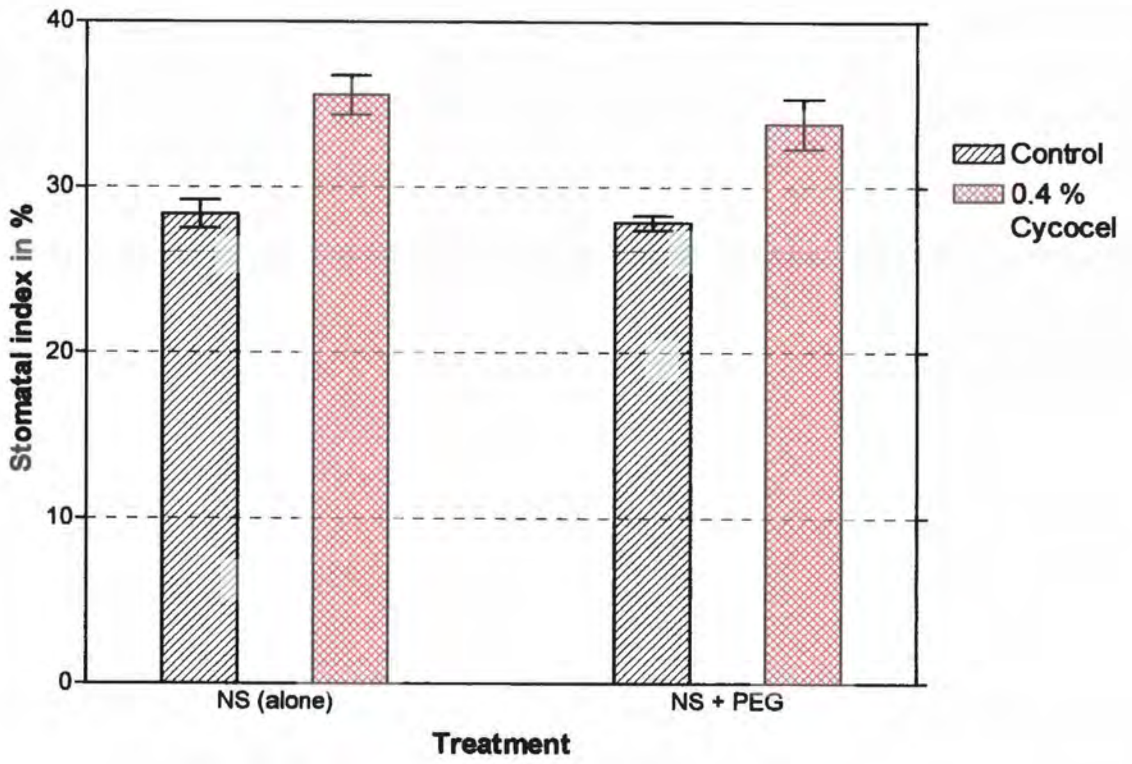
Figure 49

Stomatal Index measured in percent, as seen in the 4th leaf of wheat seedlings. Caryopses of wheat were germinated in 0.4 % of Cycocel. Then seedlings were grown in NS and NS + PEG solution.

NS = Hoagland Nutrient Solution.

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol.

Figure 49



3.3 SECTION III: A) POT EXPERIMENTS

3.3.1 Second Generation Plants (GEN2)

As has been shown in the previous sections plant development is mainly identified by the morphological changes that occur during the development of different plants parts. The growth and development of wheat seedlings was greatly affected by the pre-treatment of caryopses with Cycocel, and as stated earlier this pre-treatment was concomitant with the development of shorter and darker green plants. Most of the initial experiments on the GEN1 plants were a study of short-term effects of Cycocel. However, plants which were allowed to recover from water stress treatment were maintained in the long-term to the extent that they reproduced and produced viable caryopses to give GEN2 plants. This situation provided a suitable basis for studying long-term effects of Cycocel on wheat plants of generations subsequent to those pre-treated, i.e. any carryover effects of treatment could be investigated.

3.3.2 Initial Observations

Pre-germinated seedlings (caryopses germinated in 0.4 % Cycocel) were grown in soil in pots, and watered every 2-3 days as a recovery from the drought stress condition, as described in Methods. It was found that Cycocel pre-treatment of caryopses was able to delay the subsequent wheat inflorescence development, and also prolong the reproduction of axillary shoots to the second year. From these initial observations, the carryover effect of Cycocel pre-treatment of caryopses was further investigated through the development of a second generation (GEN2). GEN2 seedlings were derived from caryopses from the GEN1 generation, and these were not

exposed to any chemical manipulation themselves. As seen from Table 9, when these plants were subjected to water withholding treatment for about 15 days after the full emergence of the 5th leaf, plants showed some enhanced ability to retain water above that of the control plants. GEN2 plants derived from treated GEN1 plants held under a 2-day water regime had 2.95 % available water whereas those under 7-day water had 18.27 %. This compares with 2.41 % and 13.08 for the GEN1 controls respectively.

3.3.3 Carryover Effect of Cycocel on GEN2 Seedling Growth in Combination with different Watering Regimes

The length of each leaf blade of GEN2 plants (caryopses germinated in water) showed a consistent growth pattern up to the mature 4th leaf (Figure 50). As indicated by the analysis of variance there was no statistical significant difference between the leaf growth and development under both 2 and 7-day watering intervals. It appeared that the growth of all successive leaf, up to the 4th, was not effected by water limitation of 7-day watering regime, unlike the response of GEN1 seedlings (Section 1). These GEN2 plants, from Cycocel treated GEN1 samples, appeared not to be sensitive to water limitation in terms of overall growth. A potential carryover of Cycocel treatment into the GEN2 was indicated by these results.

Table 9

The influence of Cycocel, water withholding and the combination of both on the ability of GEN2 wheat seedlings to retain water after 15 days lack of water.

* 2 days = plants were watered every 2 days until the emergence of 5th leaf.

* 7 days = plants were watered every 7 days until the emergence of 5th leaf.

GEN1 = refer to the plants from commercial wheat caryopses.

GEN2 = refer to plants from the caryopses that were harvested from GEN1 plants.

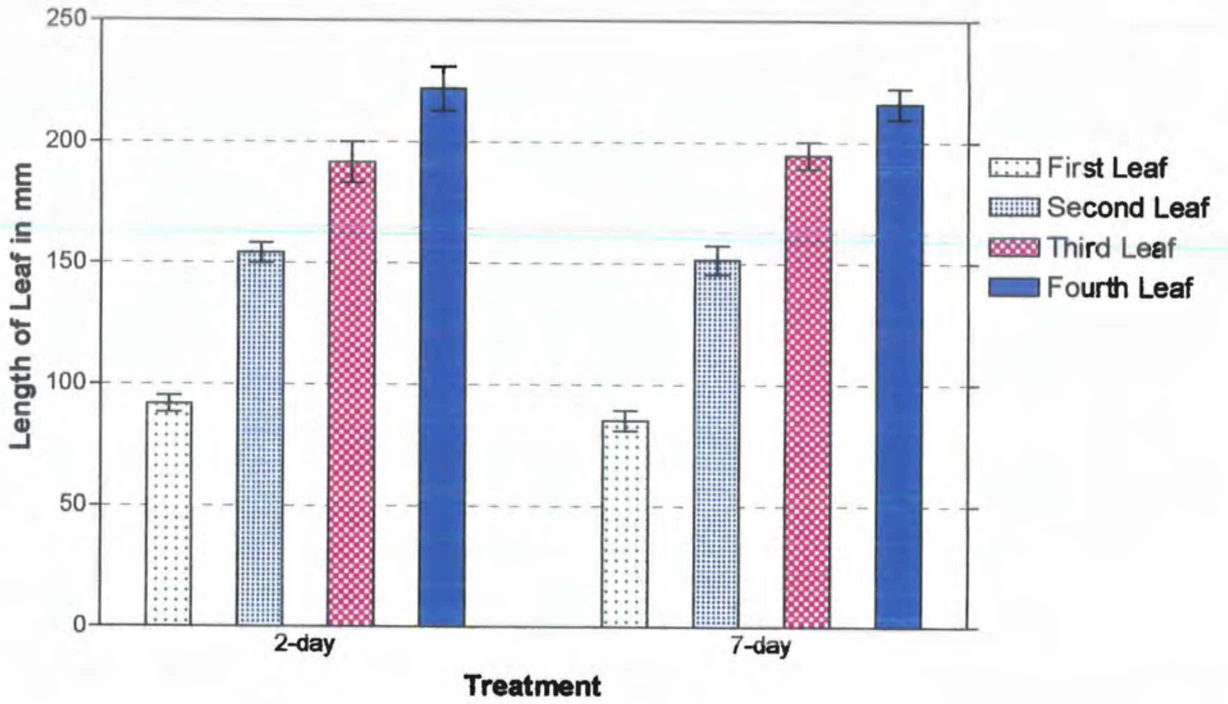
Table 9

		2 days *	7 days *
Type	Treatment	% water available	% water available
Plant:			
GEN1	Control	2.41	13.08
GEN2	-	2.95	18.27
Soil			
GEN1	-	9.65	13.90
GEN2	-	10.85	12.54
Soil at field capacity		88.65	

Figure 50

Length of individual leaves of GEN2 seedlings. Caryopses germinated in water alone. Plants were subjected to 2 and 7-day watering regimes under pot growth conditions.

Figure 50



3.3.4 Pigmentation

As previously indicated for GEN1 plants Cycocel did alter chlorophyll levels in 3rd and 4th leaf of the wheat seedlings. From Figure 50 it can be seen that GEN2-seedlings indicated some resistance to water limitation. Therefore it was essential to determine whether or not the chlorophyll levels were maintained in GEN2 seedlings. This particular study examined the level of chlorophyll in 3rd and 4th leaf of GEN2 seedlings grown under two watering regime intervals, as previously described.

The 3rd leaves of GEN2 plants contained slightly more total chlorophyll and individual chlorophyll a and chlorophyll b than did GEN1 plants when maintained under a 2-day watering regime. When the GEN1 plants were subjected to a 7-day watering interval regime from the coleoptile stage to the maturity of the 3rd leaf there was a statistical significant ($P = 0.05$) decrease in the total chlorophyll accumulation (Figure 51). In contrast when GEN2 plants were subjected to the same treatment then no decrease in chlorophyll level was seen. This indicates that Cycocel pre-treatment of the GEN1 generation resulted in a carryover into the GEN2 of the ability to withstand the effect of water limitation as measured by chlorophyll content.

Individual chlorophyll a and chlorophyll b showed the same pattern of response as did total chlorophyll (Figure 52 and 53). As was seen in Section 1 Cycocel pre-treatment gave no differential effect on the two photosynthetic systems.

Here again it can be seen from Figure 54 that the 4th leaves of GEN2 plants accumulated significantly higher total chlorophyll as well as chlorophyll a and chlorophyll b than did GEN1 plants when maintained under both 2 and 7-day watering regimes. However, when the GEN1 plants were subjected to a 7-day watering

Figure 51

Total chlorophyll accumulation in 3rd leaves of GEN2-plants, when grown under a 2 and 7-day watering regime.

Figure 51

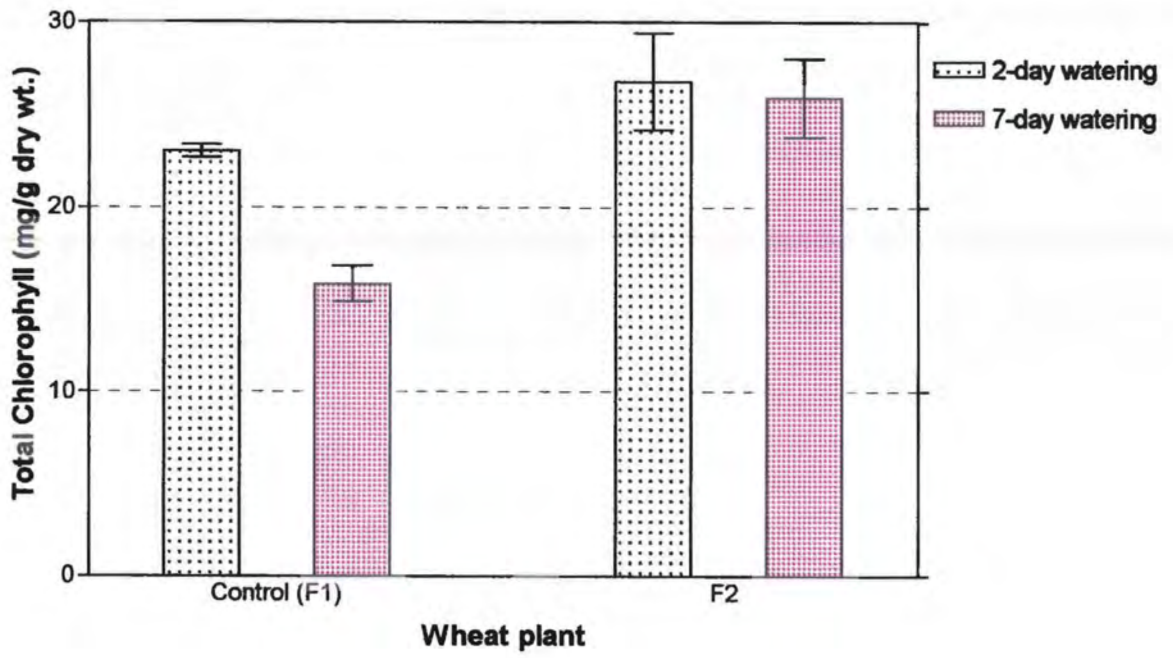


Figure 52 & 53.

Chlorophyll a and chlorophyll b accumulation in 3rd leaves of GEN2 plants, when grown under a 2 and 7-day watering regimes.

Figure 52

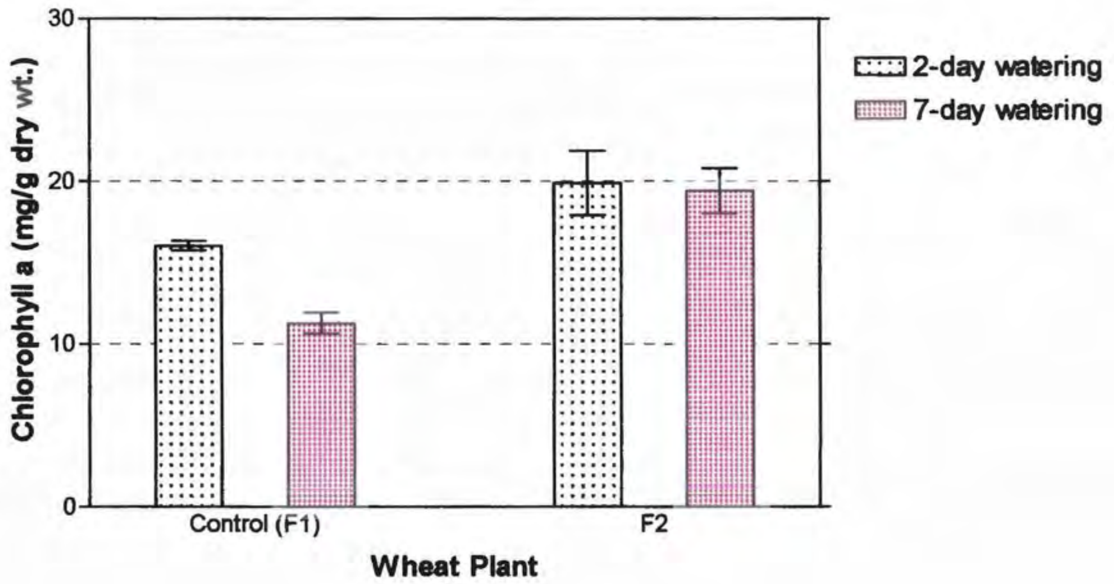


Figure 53

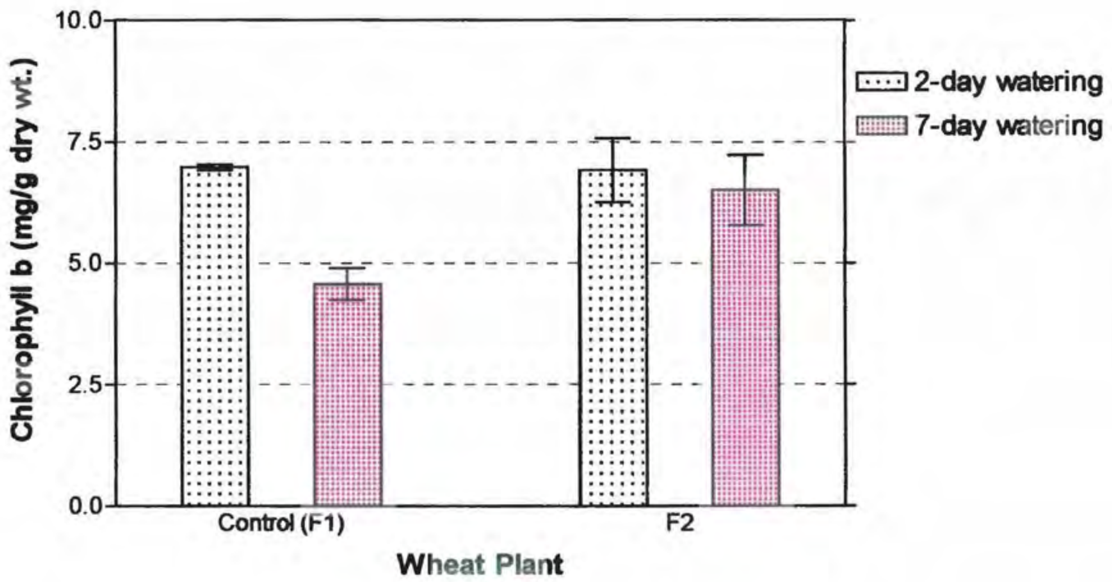
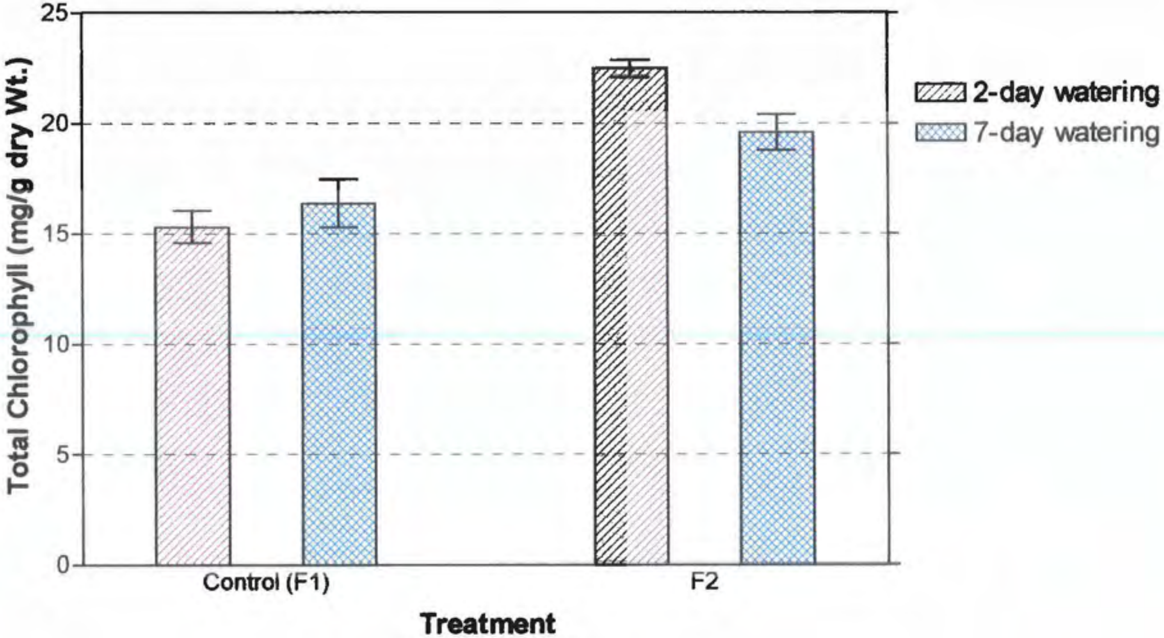


Figure 54

Total chlorophyll accumulation in the 4th leaves of GEN2-plants, when grown under a 2 and 7-day watering regime.

Figure 54



from the coleoptile to the maturity of the 4th leaf an inconsistency of chlorophyll accumulation was observed whereas in the 3rd leaf chlorophyll was reduced under the water limitation treatment, the 4th leaf did not show this response. There was no significant difference between the two watering regimes. It was also seen that individual chlorophyll a and chlorophyll b showed essentially the same pattern of response as for the total chlorophyll (Figure 55 and 56).

As with the 3rd leaf it appeared that Cycocel pre-treatment of caryopses was able to enhance chlorophyll accumulation in the 4th leaf. This carryover effect was also seen when the plants were subjected to the 7-day watering intervals. However, when chlorophyll was extracted from the GEN2 tissue, by the use of 80 % acetone, as described in Methods a proportion of the chlorophyll was left behind in the 4th leaf but not in the 3rd leaf. It was extracted completely from both leaves of the GEN1 generation. In order to release the total chlorophyll from GEN2 leaves it was necessary to grind the tissue in 80 % acetone after subjecting these to the normal extraction in 80 % acetone. Figure 57 shows the chlorophyll levels extracted with and without grinding. Approximately equal amounts of chlorophyll were extracted by both methods and together gave values comparable to the total chlorophyll extracted from GEN1 plants using acetone alone. Water limitation treatment did not have any effect here.

The chlorophyll retention in tissue may be attributable to a Cycocel pre-treatment effect on the tissue cell wall/membrane. These observations suggested that anatomical changes could be taking place in tissues in response to Cycocel pre-treatment preventing release of chlorophyll. Their effects could also be related to lack of wilting.

Figure 55 & 56

Chlorophyll a and chlorophyll b accumulation in 4th leaves of GEN2 plants, when grown under a 2 and 7-day watering regimes.

Figure 55

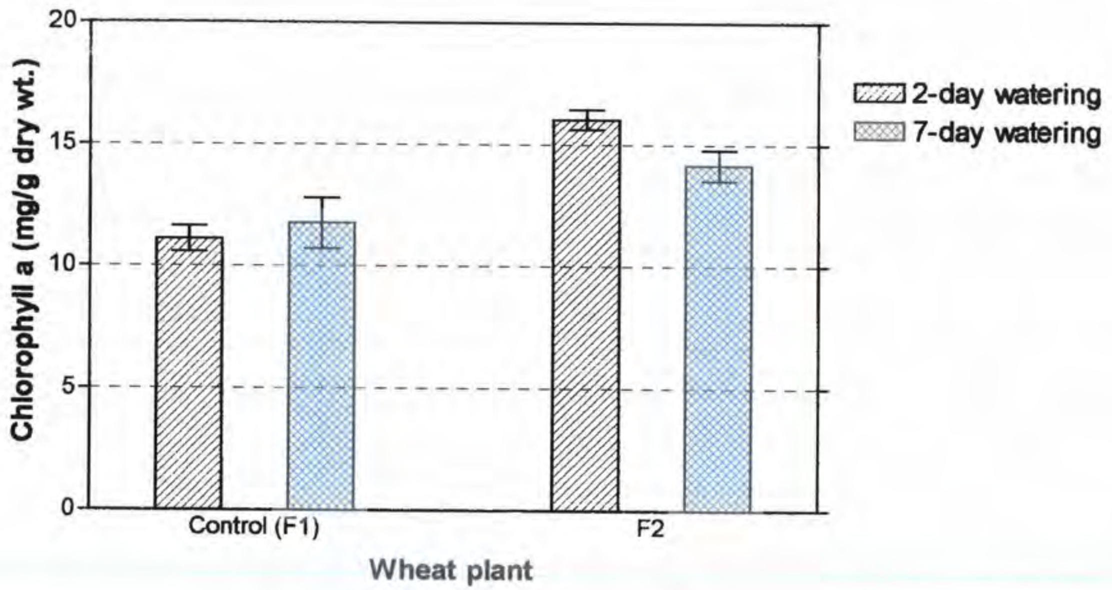


Figure 56

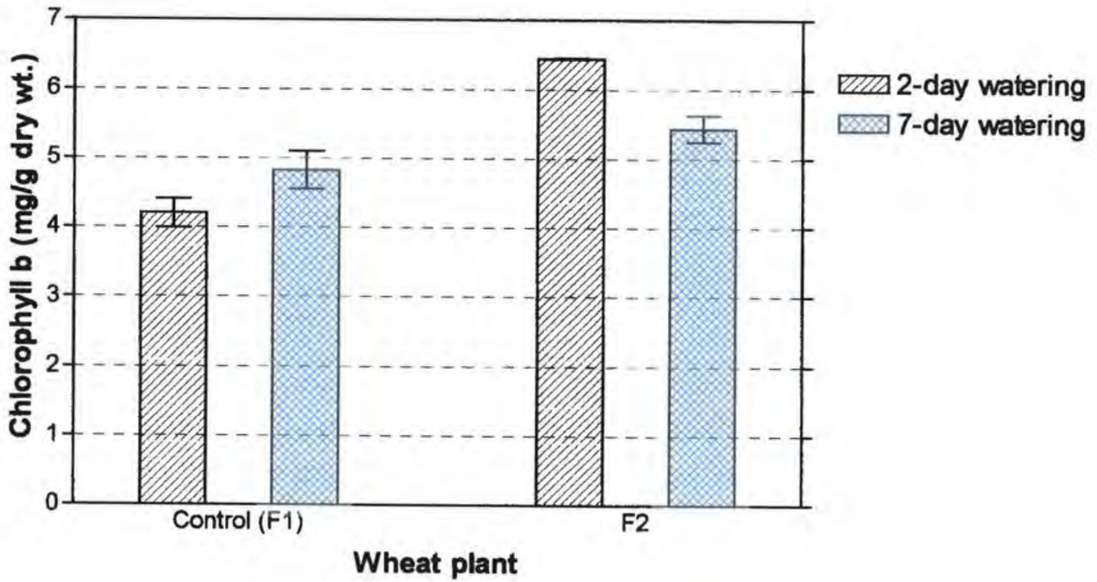
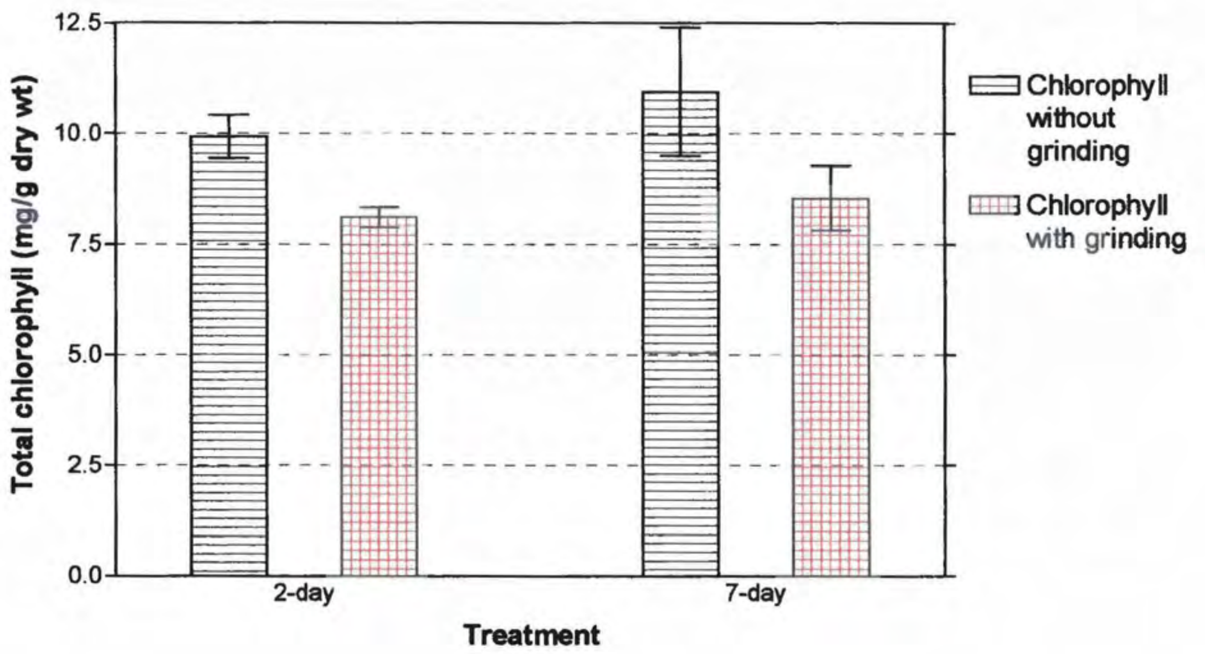


Figure 57

Chlorophyll extracted by treating 4th. leaf of GEN2 plants , when grown under a 2 and 7-day watering regimes, with 80% acetone (green) and by subsequently grinding the same leaf tissue sample in 80% acetone (red).

Figure 57



An anatomical investigation was made of wheat leaves to localise the chlorophyll present. Transverse sections from the 4th leaf tissue of the wheat plants were stained with calcofluor, and viewed under the illumination of the fluorescence microscope. In order to check the presence of chlorophyll in the leaf tissues a blue-violet block filter was utilised in order to enhance the fluorescence of chlorophyll, as described in the Methods. These analyses were undertaken after the extraction of chlorophyll with 80 % acetone, as in Methods.

The transverse section of the wheat leaf (Control GEN1), sampled from 2-day watering regime plants, showed a slight chlorophyll presence in the cells of the leaf tissues, as indicated by the red colour illumination of the cells (Plate 4). This residual chlorophyll was not apparent when cells were viewed under visual bright illumination where the tissue had a yellow coloration. It was also noted that the cells of the leaves collapsed after the chlorophyll acetone extraction and the tissue were distorted.

In contrast, leaf tissue for GEN2 plants, from Cycocel pre-treatments, did not show the same features as the GEN1 control tissues (Plate 5 and 6). In specimens of both the 2 and 7-day watering regimes it was seen that leaf cell structure appeared fully turgid, the cells did not collapse and the tissue was not distorted. This suggests that here cell wall structure was more rigid to withstand wilting. In addition a very bright red fluorescence was seen in the cells indicating the presence of chlorophyll. This confirmed the extraction results and was consistent with the green coloration of the tissues. Cycocel appears to influence chlorophyll retention in the cells in response to acetone extraction. It is possible that the solvent was prevented from penetrating into the cells by cell wall changes or that their wall properties prevented the exit of the chlorophyll in solution.

Plate 4

Transverse section of the GEN1 wheat leaf, sampled from the 2-day watering regime, after the 80 % acetone extraction. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m.

Plate 4

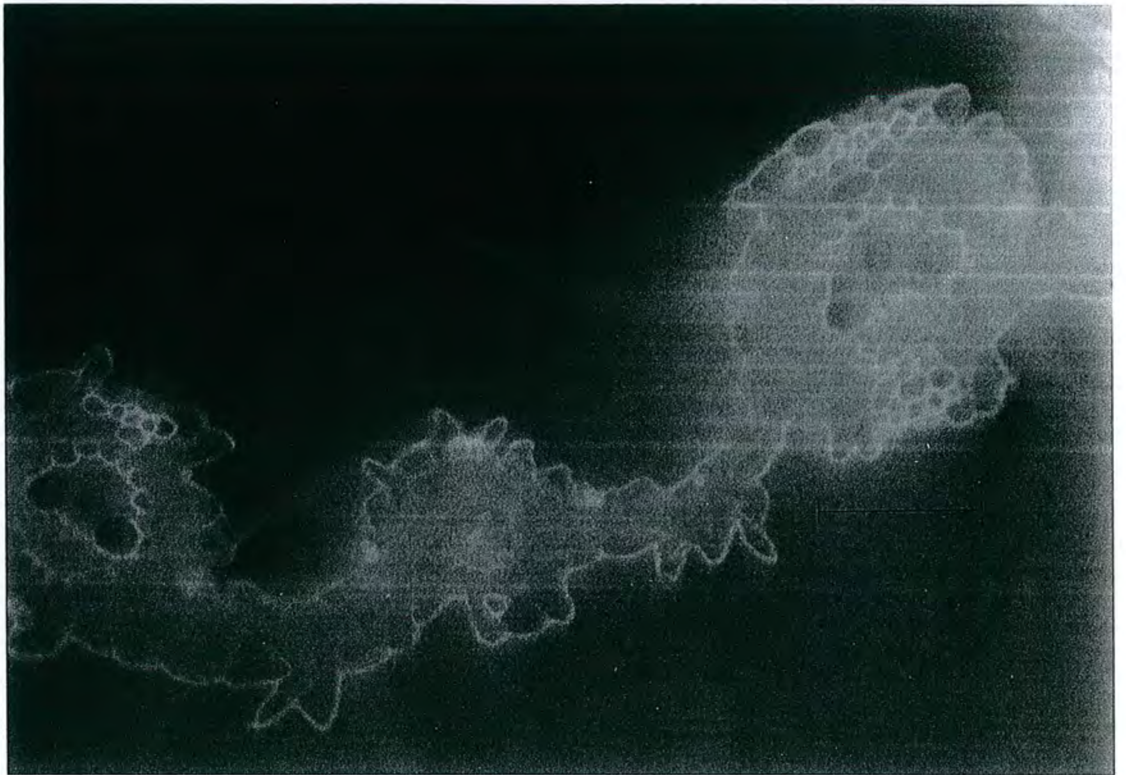


Plate 5

Transverse section of the GEN2 wheat leaf, sampled from the 2-day watering regime, after the 80 % acetone extraction. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m.

Plate 6

Transverse section of the GEN2 wheat leaf, sampled from the 7-day watering regime, after the 80 % acetone extraction. Unstained specimen mounted in water, viewed and photographed under the illumination of the fluorescence microscope at a magnification of X40 with a blue-violet filter. Chlorophyll is fluorescing red, cutinised and lignified cells are fluorescing yellow. Abbreviations: ad = adaxial surface; ab = abaxial surface; ue = upper epidermis; le = lower epidermis; m = mesophyll; vb = vascular bundle; xy = xylem; ph = phloem; st = stomata. Scale bar = 100 *u*m.

Plate 5

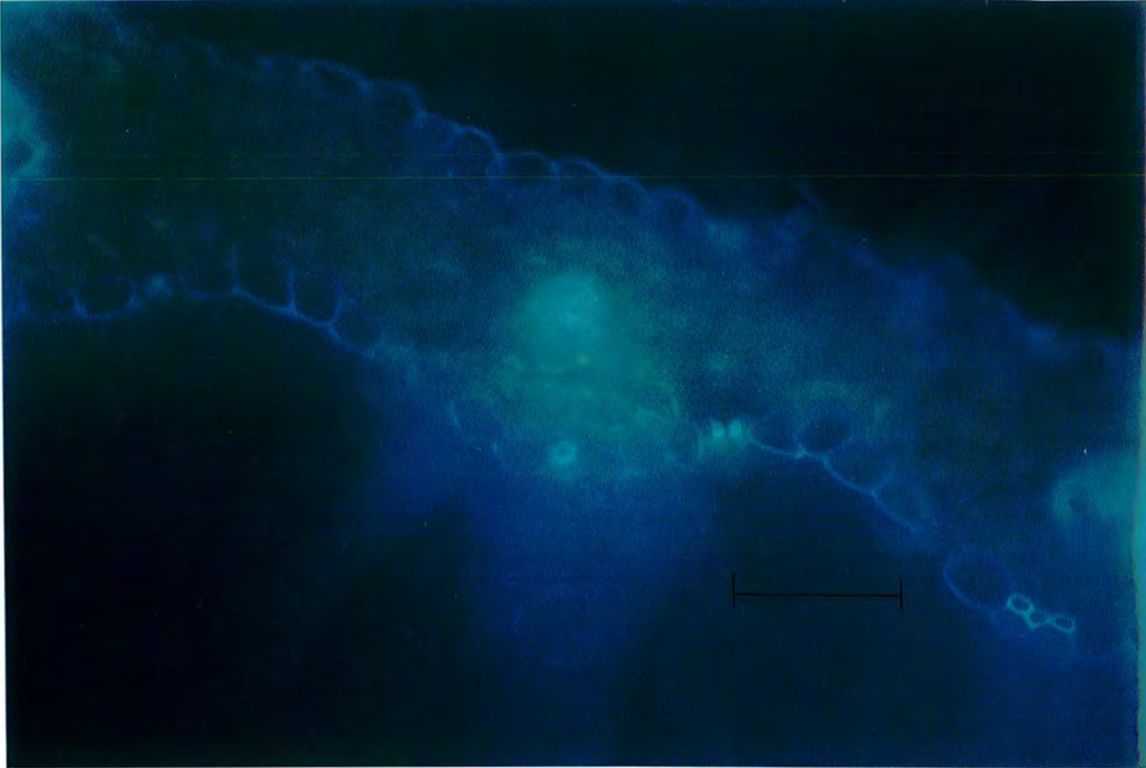
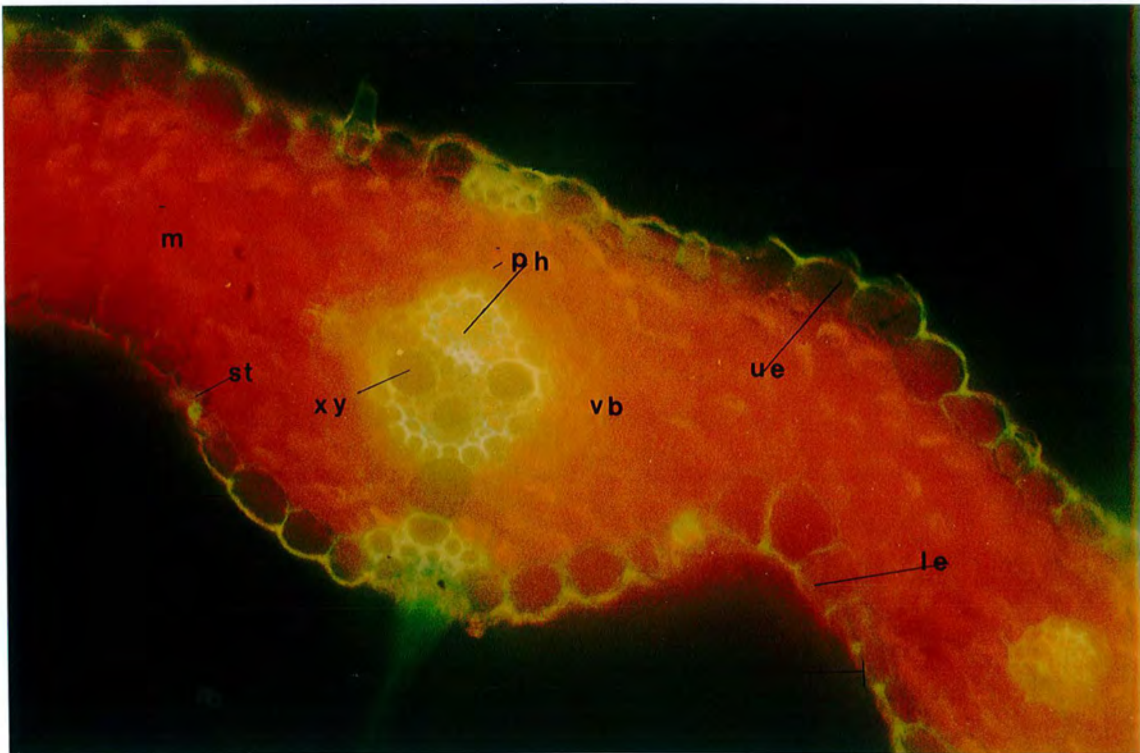


Plate 6



(X)

3.3.5 Stomata

It is commonly known that water limitation results in stomatal closure. Earlier observation on water retention, carried out on the GEN2 plants, indicated that Cycocel pre-treatment of caryopses seems to have influenced the water availability in GEN2 plants over the control, GEN1 plants (Table 9).

Here it was of interest to investigate the potential carryover effect of Cycocel pre-treatments on stomatal characteristics related to water limitation: the length of stomatal pore, Stomatal Density and index, and the epidermal cell of the 3rd and 4th leaf of GEN2 plants grown under 2 and 7-day watering regimes.

The stomatal pore length values shown in Figure 58 indicate a consistent pattern for both 3rd and 4th leaves grown under 2 and 7-day watering treatments. Stomatal length in the 4th leaf was significantly decreased over the 3rd leaf in both treatments (P = 0.05). This indicates some variability in stomatal characteristics with individual leaves of the same plant. It was also observed that plants grown under 7-day water limitation condition did not show altered stomatal pore length for either leaf. Therefore watering regime did not affect the stomatal length in the GEN2 plants compared with GEN1 controls. This similarity in pattern length and its persistence under water limitation may be initiated by the changes caused, in GEN1 plants, by the Cycocel pre-treatments of caryopses.

As seen from Figure 59 there was no statistical difference (P = 0.05) in the 3rd and 4th leaf Stomatal Density when GEN2 plants were grown under 2 and 7-day watering regimes. Stomatal Density of the 3rd leaf was seen to increase slightly when plants were subjected to 2-day watering regime, but was statistically not significant over the 7-day watering regime. In contrast, the 4th leaf was seen to have a consistent

Figure 58

Stomatal pore length in µm, as seen in the 3rd and 4th leaves of GEN2 seedlings. Caryopses of wheat were germinated in water. Plants were subjected to 2 and 7-day watering intervals.

Figure 58

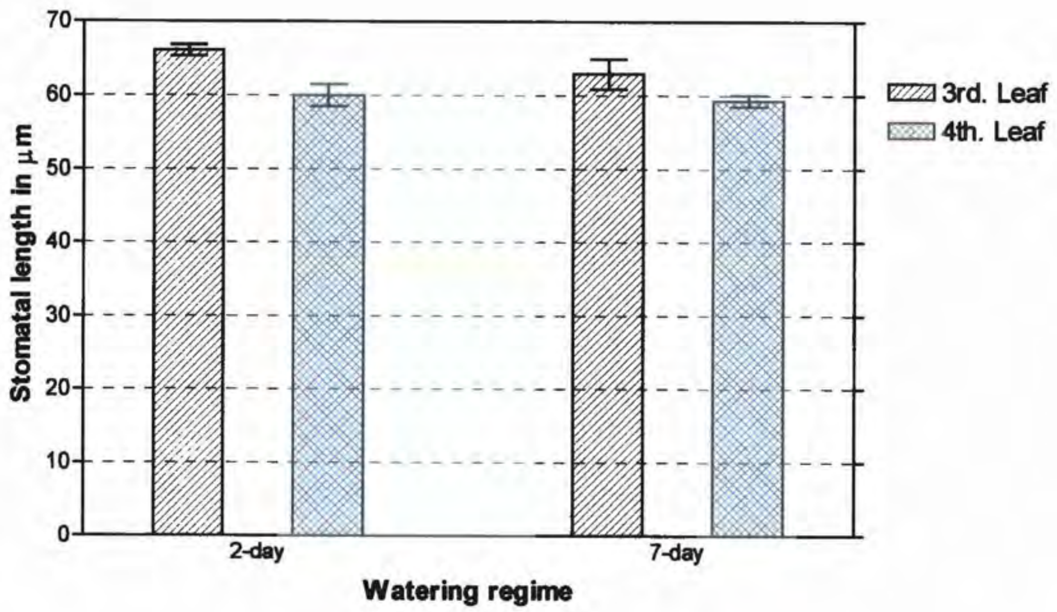
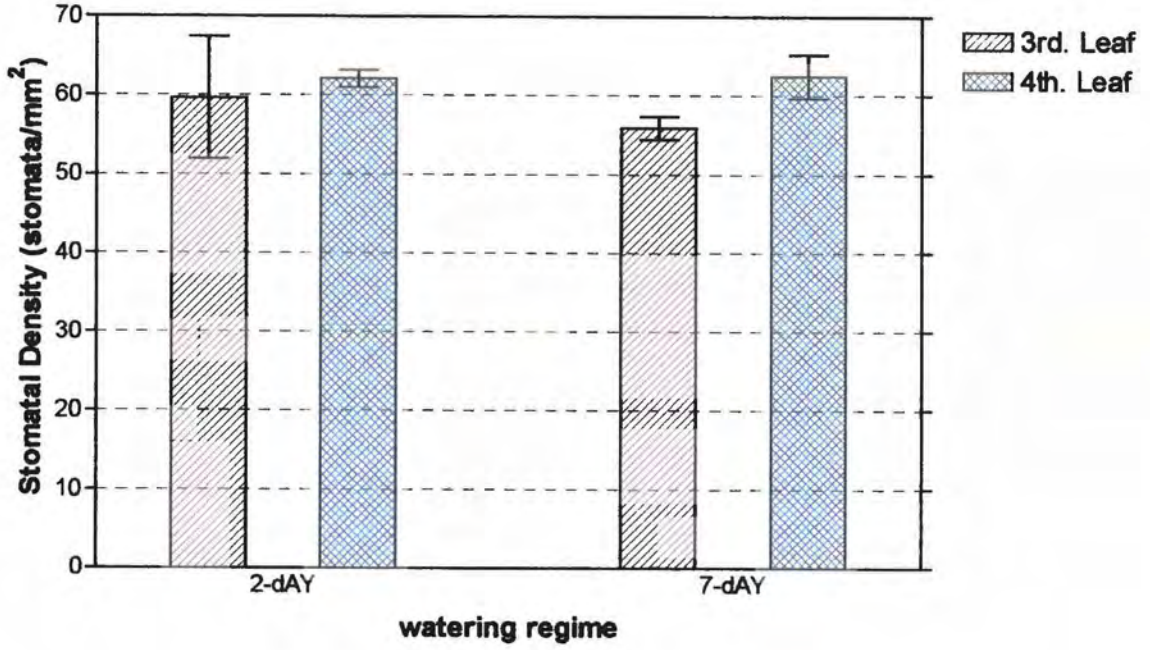


Figure 59

Stomatal Density measured as stomata/mm^2, as seen in the 3rd and 4th leaves of the GEN2 seedlings. Caryopses of wheat were germinated in water. Plants were subjected to 2 and 7-day watering intervals.

Figure 59



pattern of stomatal length under both treatments. Therefore neither the 7-day nor the 2-day treatment resulted in any alteration of the Stomatal Density of the 4th leaf.

As indicated from Figure 60 that the Stomatal Index of 3rd and 4th leaf of GEN2 plants shows no statistical significant ($P = 0.05$) under both 2 and 7-day watering regimes. It was found that Stomatal Index values of 3rd leaves were almost identical for GEN2 plants subjected to both watering treatments. In contrast, when the Stomatal Index of the 4th leaf was measured, it was found that the GEN2 plants maintained under 2-day watering regime showed a reduced stomatal number, but this was not statistically significant ($P = 0.05$). However, when the GEN2 plants were subjected to 7-day water limitation there was a slight increase in Stomatal Index of 4th leaf. Therefore this suggested that watering did increase the stomatal number of the 4th leaf but was not statistically different from the 3rd leaf.

The difference in epidermal cell density was also found to be not significant for the 3rd and 4th leaves of the GEN2 plants under both treatments (Figure 61). The 3rd and 4th leaves, when subjected to 2-day watering, appeared to have a slightly increased the epidermal cell density, but this was not significant. Here again it can be seen a consistent pattern of epidermal cell occurrence in the 3rd and 4th leaves under watering limitation.

From these results it appears that water regime has a minimal effect, if any, on stomatal characteristics of GEN2 plants derived from Cycocel treated caryopses. Therefore any limitation to water loss could not be accounted for by stomatal characteristics recorded here.

Figure 60

Stomatal Index measured in percent, as seen in the 3rd and 4th leaves of the GEN2 seedlings. Caryopses of wheat were germinated in water. Plants were subjected to 2 and 7-day watering intervals.

Figure 60

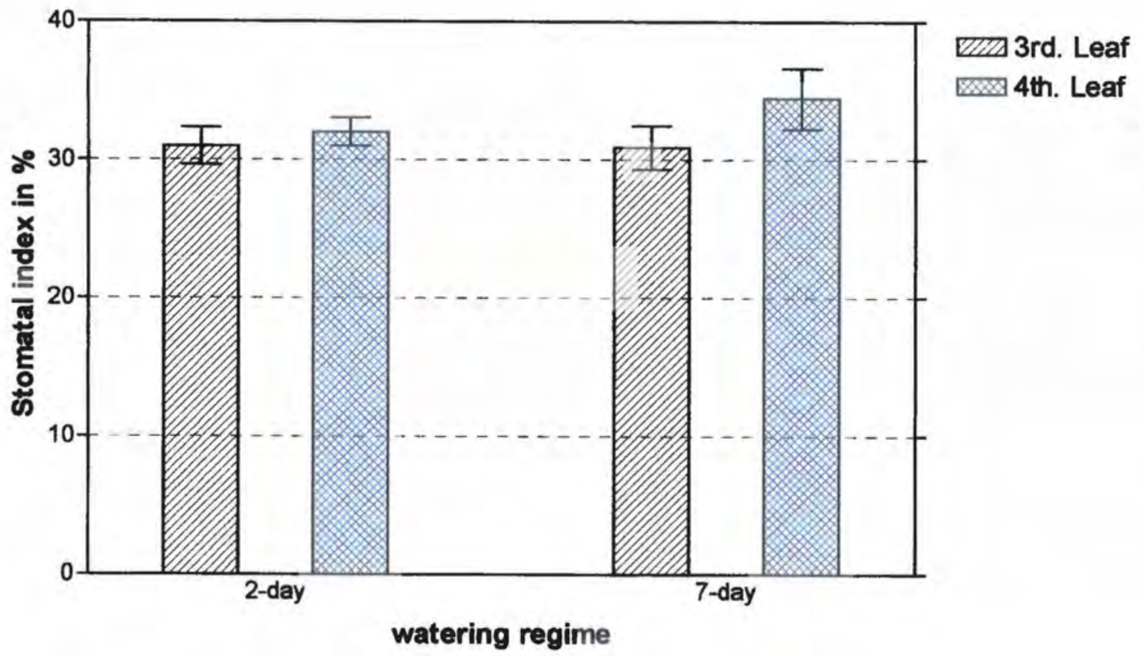
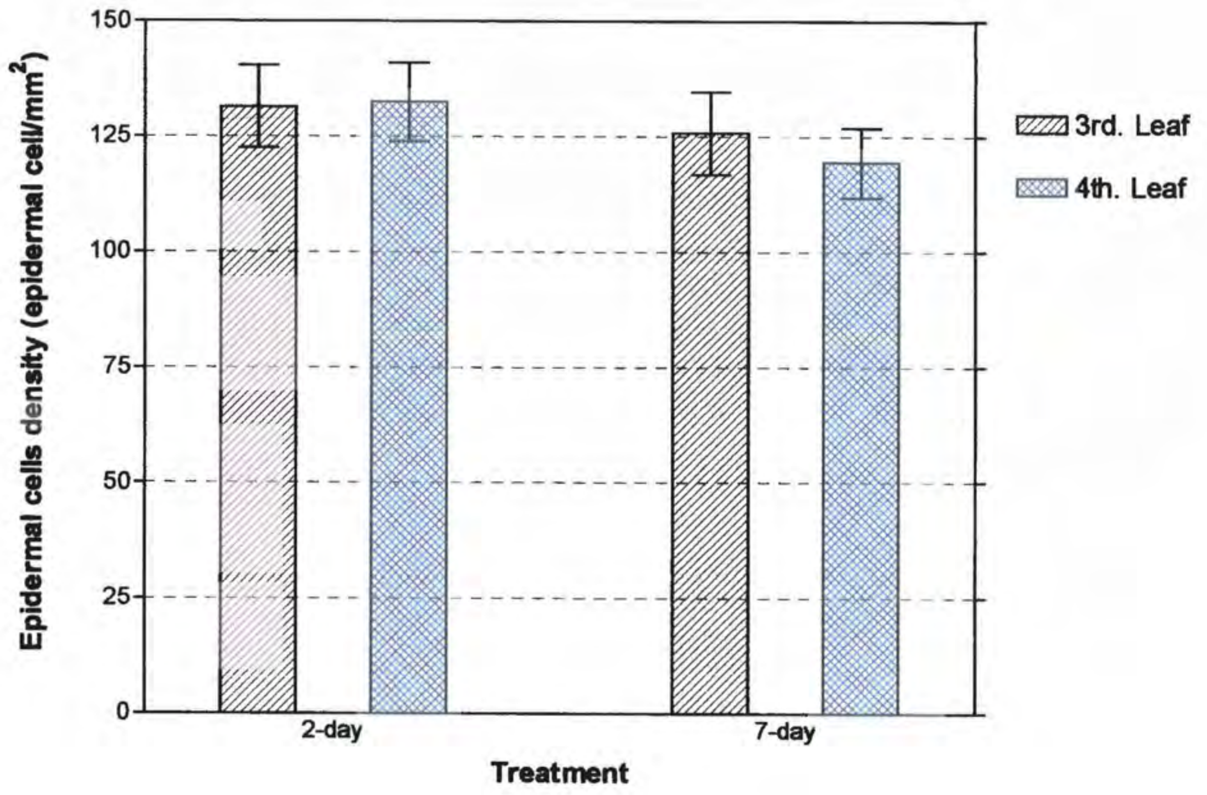


Figure 61

Epidermal cells density measured as epidermal cell/mm^2, as seen in the 3rd and 4th leaves of the GEN2 seedlings. Caryopses of wheat were germinated in water. Plants were subjected to 2 and 7-day watering intervals.

Figure 61



B) Hydroponic Experiments

Here again the need for a finer control of water limitation treatment and access to roots was found to be necessary to recover the tissue in an intact state and free of contamination therefore sets of experiments were performed using hydroponic culture techniques.

3.3.1 Fresh and Dry Weight

Stunting of GEN1 seedlings has been recognised as one of the conspicuous effect of Cycocel pre-treatment of caryopses. Thus as stated previously the influence of long-term effect of Cycocel was investigated. Here again GEN2 seedlings were germinated in water only and grown in NS and NS + PEG at -5 bar. The plants were then harvested after 30 days of growth, fresh and dry weight were determined as in the methods for both shoots and roots.

As in section II, for the leaf tissue, whole plants were harvested so that the fresh and dry weight values represent an average for all leaves. It was noted from Figure 62 and 63 that growing GEN2 plants in NS treatment alone increased the fresh weight values of shoot, whereas the same treatment decreased the dry weight values of the same plants compared with the control seedlings GEN1 plants. However, under the PEG treatment both fresh and dry weight of the GEN2 plants and the control GEN1 plants were reduced but not statistically significant ($P = 0.05$).

Here again the analysis of fresh and dry weight of the root system from the same treatment was also determined (Figure 64 and 65). It was found that the fresh weight of the root system followed essentially the same pattern as for shoots, when grown under either NS or NS + PEG solutions. However, the dry weight values

showed a significant increase for both GEN1 and GEN2 plants when grown in the NS + PEG solutions compared with the NS alone. No significant difference was found between the response of the two sets of tissue. This effect appears to be related to potential accumulations of more dense cells under PEG treatment where water uptake limitation is reflected in the fresh weight values. Cycocel pre-treatment did not appear to influence this response.

Figure 62

Fresh weight in gram of the GEN2 shoots, when grown in NS and NS + PEG solutions.

NS = Hoagland Nutrient Solution

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol at -5 bar.

Figure 63

Dry weight in gram of the GEN2 shoots, when grown in NS and NS + PEG solutions.

NS = Hoagland Nutrient Solution

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol at -5 bar.

Figure 62

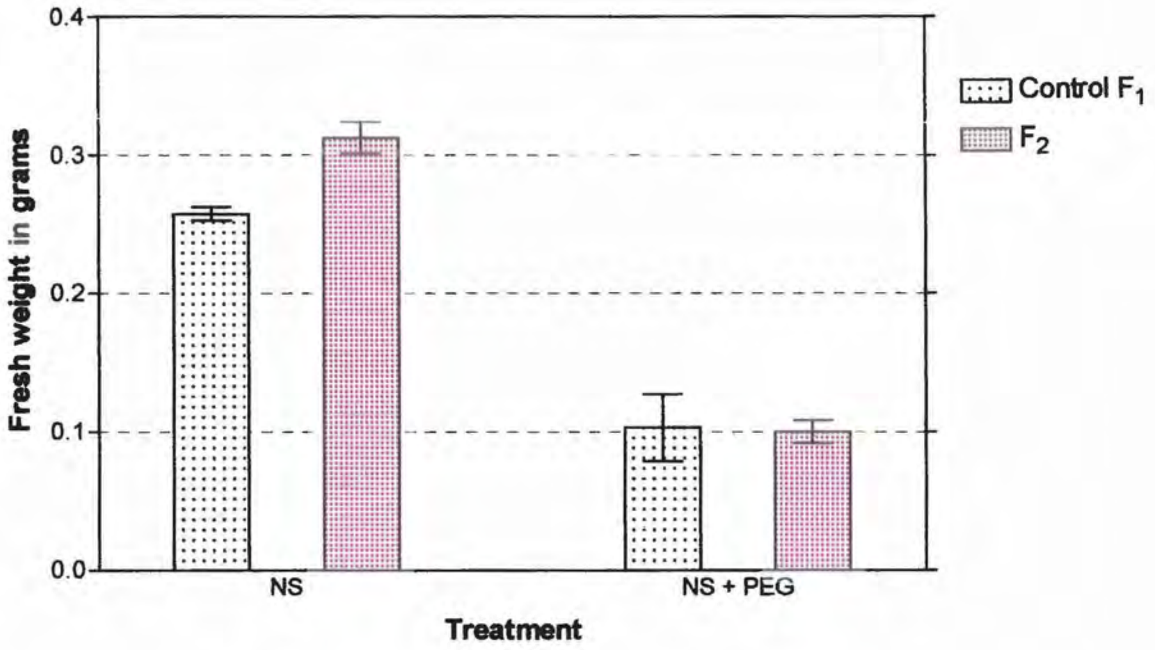


Figure 63

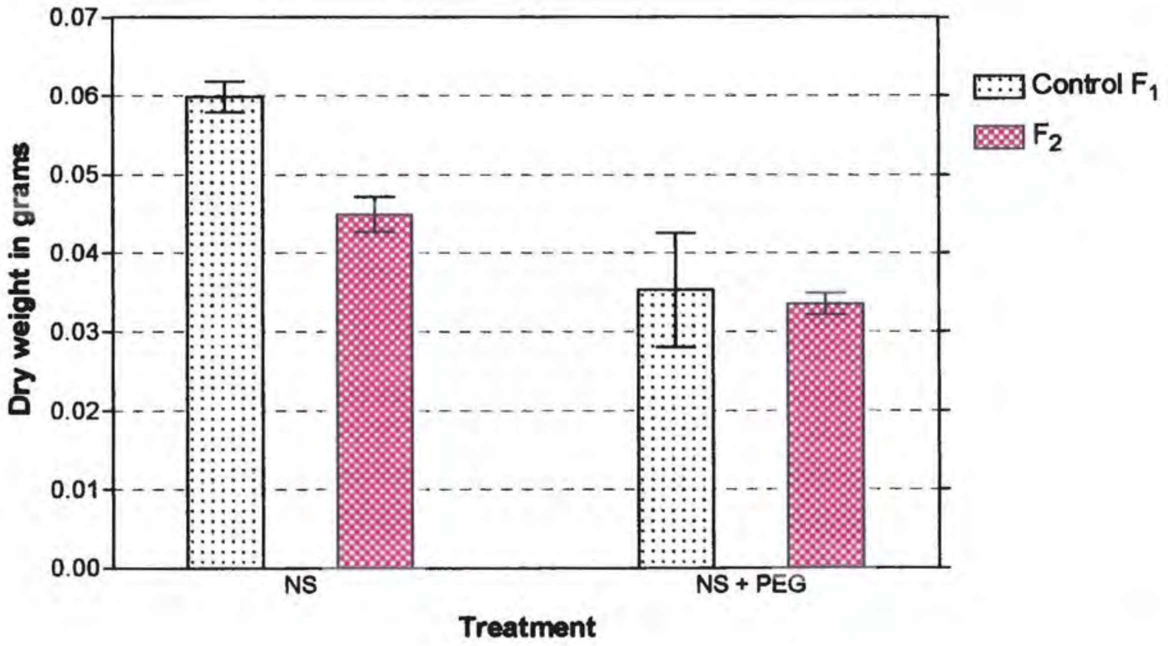


Figure 64

Fresh weight in gram of the GEN2 roots, when grown in NS and NS + PEG solutions.

NS = Hoagland Nutrient Solution

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol at -5 bar.

Figure 65

Dry weight in gram of the GEN2 roots, when grown in NS and NS + PEG solutions.

NS = Hoagland Nutrient Solution

NS + PEG = Hoagland Nutrient Solution + Polyethylene Glycol at -5 bar.

Figure 64

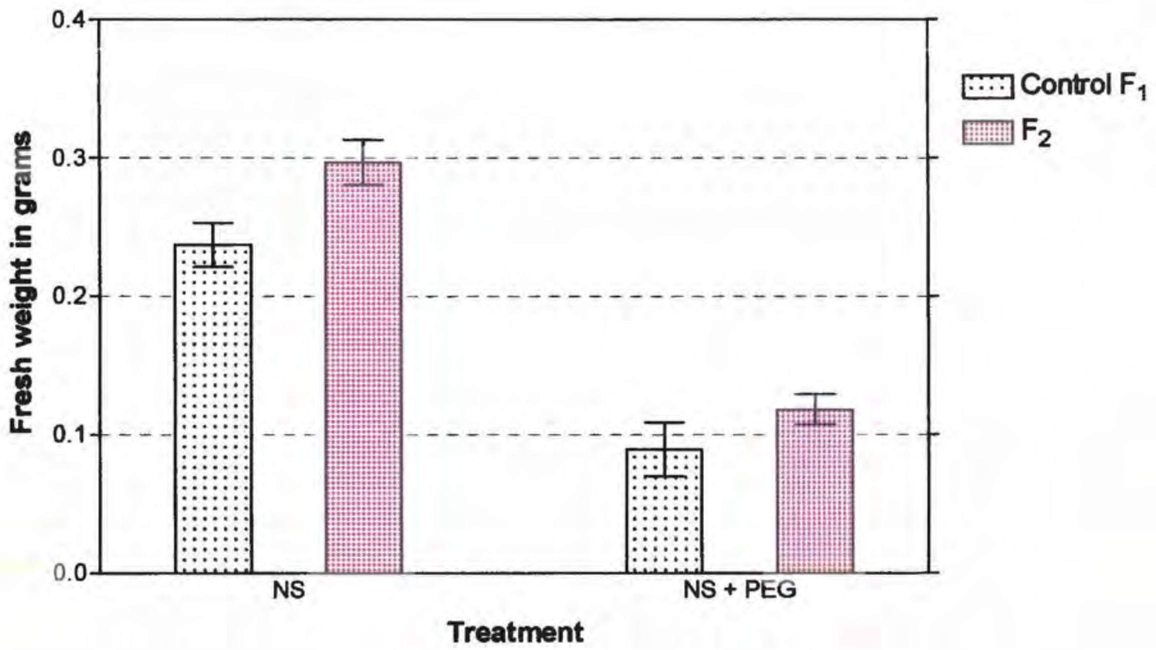
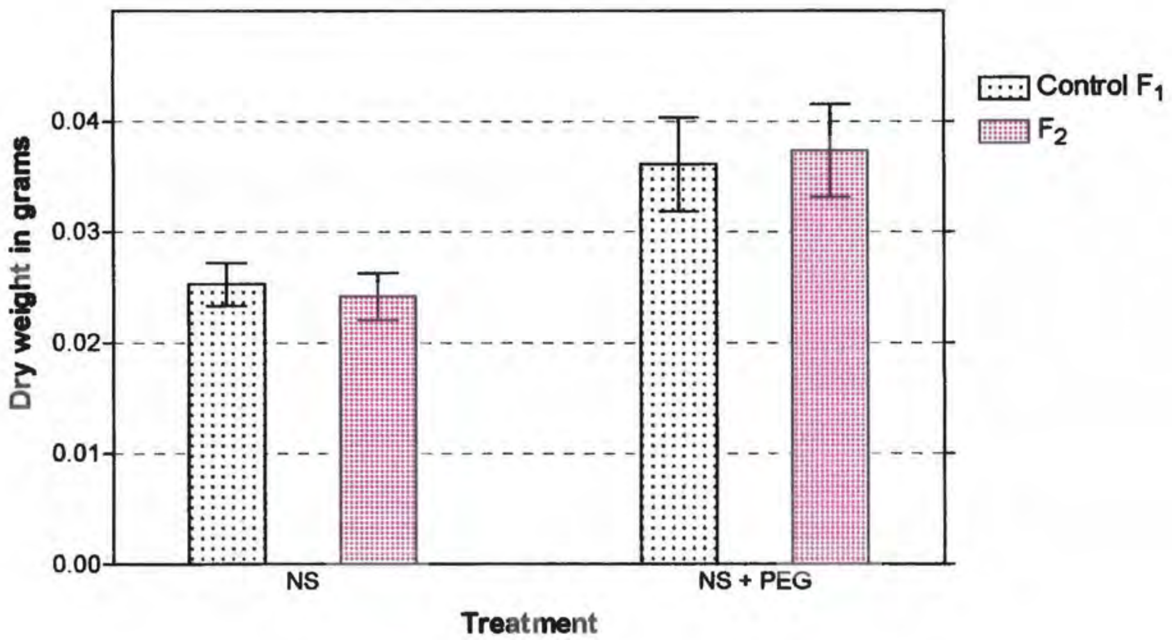


Figure 65



3.4 Anatomy

There are many recognised fluorescent substances found in plants. They are mainly found in cell walls, chloroplasts and vacuoles (Rost, 1995). In general, plant tissues can be stained with specific fluorescent dyes to induce fluorescence, such stains are acridine orange (AO) and calcofluor.

It has been stated by Rost (1995) that there are two recognised ways of staining, conventional, and vital or supra-vital. The conventional staining method is where the fluorescent dye binds directly onto the substrate (the tissue component). This can be used in a fresh or fixed tissue. The vital or supra-vital staining is the method of staining the living tissues by the mean of injecting the dye into an organism, or placing the organism in a bath containing the staining dye. Conventional staining was used in this study.

Gupta and De (1988) indicated that AO is generally used in an aqueous solution at a low concentration. Likewise, Dingle and Barret (1969) stated that AO can be used as a supra-vital dye that binds to many anionic sites, as well as those in the membranous cell organelles.

Hughes and McCully (1975) reported that the optical brightener calcofluor White M2R New is mainly used at a low concentration of 0.01 % aqueous solution to stain the cell wall of higher plants. They also stated that calcofluor can be used as a vital dye for intact plants or for hand cut sections, in which case it binds strongly to cellulose, carboxylated polysaccharides, and callose.

Combining the observations obtained from the previous physiological study on lack of wilting, water retention and chlorophyll retention, there were indications that changes in the anatomy of the tissues could be important in the Cycocel response. Therefore an anatomical investigation was initiated. Potential effects of Cycocel

included cuticle thickness changes, cell walls deposition and cell size related to stomatal patterns.

3.4.1 Leaf tissue

Cell wall thickness and structural rigidity in the leaf tissues was compared for samples of leaf tissue in combination with 2 and 7-day watering regimes and Cycocel pre-treatment. In Plates 7, 8, 9 and 10 the fluorescence produced by Calcofluor, from the leaf tissues of the GEN1 and GEN2 plants, were observed using an ultraviolet block filter (UV). It can be seen from Figures 7 and 8 that the cell walls of the control GEN1 tissues fluoresced but there was no obvious difference to be seen in the wall thickness of the epidermal cells in particular. No obvious cuticle was observed in the tissues for comparison between the two watering regimes.

However, it can be seen from Figures 9 and 10 that the cell wall of GEN2 plants, from Cycocel pre-treatment, did fluoresce under the UV barrier filter, cell walls did not collapse, and the wall structure appeared to be more rigid.

A further analysis of anatomy was performed on the whole length of leaf blades for an examination of sections taken from the tip, mid and base regions to investigate any influence of Cycocel. Here chlorophyll accumulation and cell wall thickness of the non-extracted whole leaf blades of the GEN2 plants were examined in combination with 2 and 7-day watering intervals.

From Plates 11, 12, 13, 14, 15, and 16, it can be seen that there was no obvious differences in the chlorophyll present in the tip, mid, and base segments of the 2-day watering regimes of the GEN2 plants when compared with the control GEN1 leaf

Plate 7

Transverse section of the GEN1 wheat leaf, sampled from the 2-day watering regime, after the 80 % acetone extraction. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m.

Plate 8

Transverse section of the GEN1 wheat leaf, sampled from the 7-day watering regime, after the 80 % acetone extraction. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m.

Plate 7

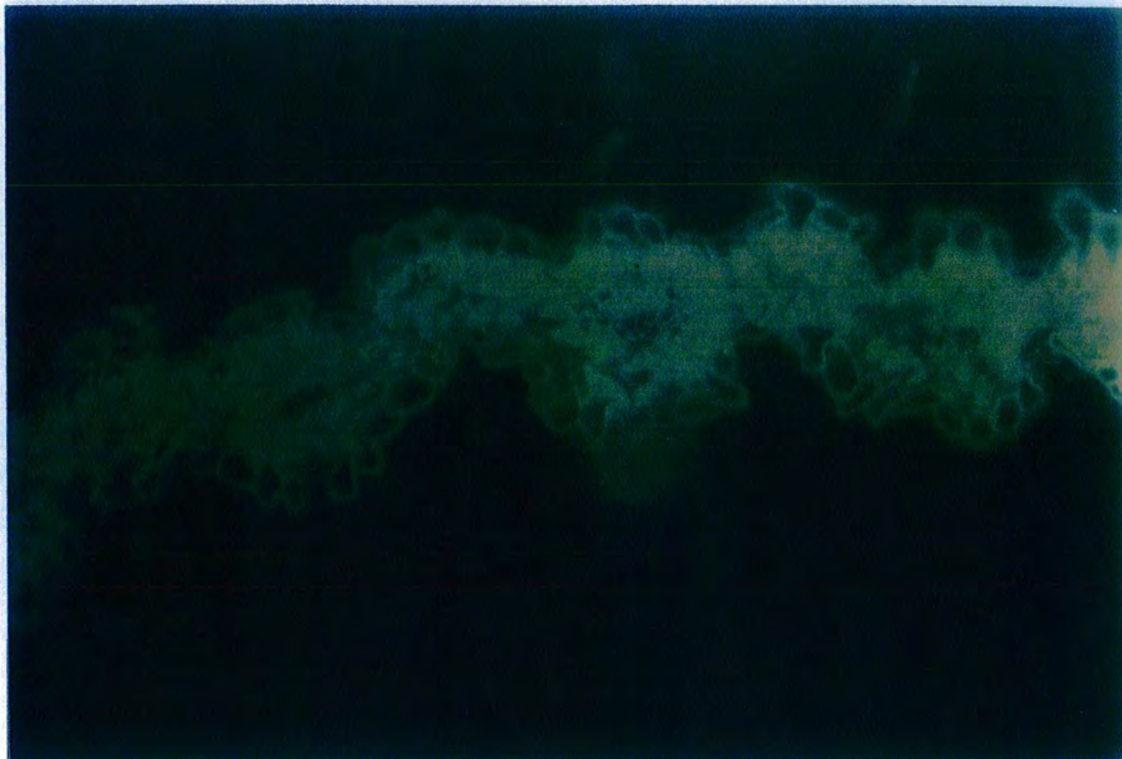


Plate 8

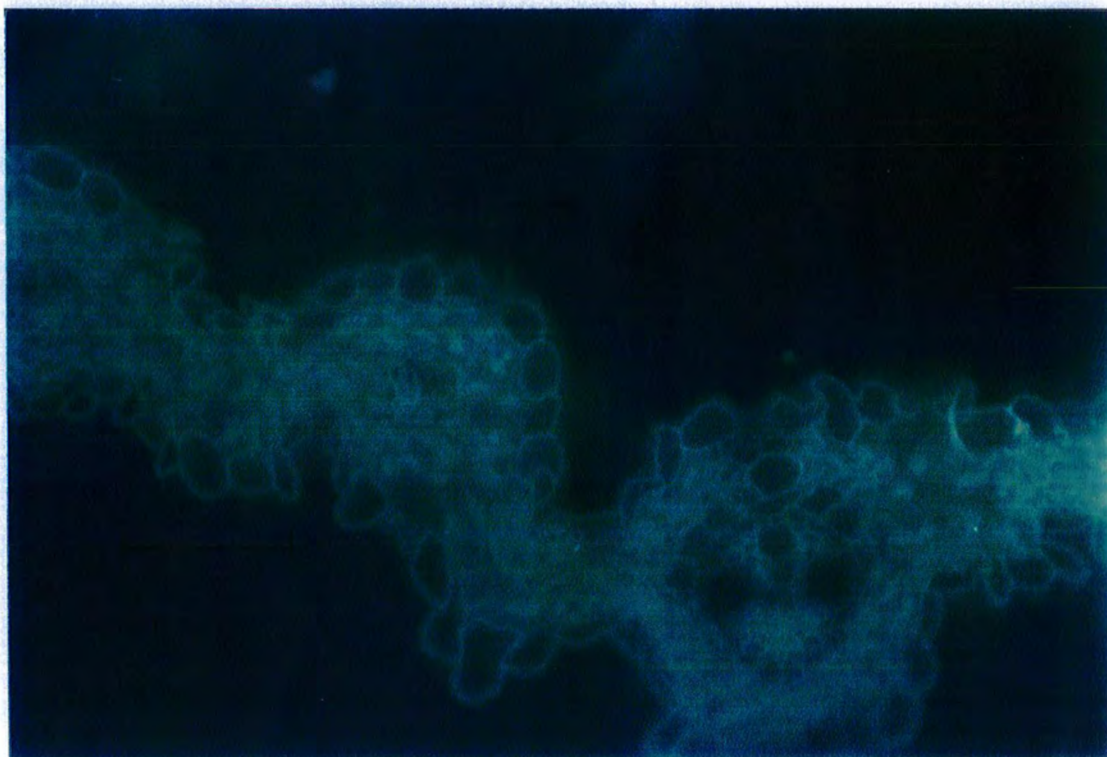


Plate 9

Transverse section of the GEN2 wheat leaf, sampled from the 2-day watering regime, after the 80 % acetone extraction. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m.

Plate 10

Transverse section of the GEN2 wheat leaf, sampled from the 7-day watering regime, after the 80 % acetone extraction. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X40 with an ultraviolet filter. Scale bar = 100 *u*m.

Plate 9

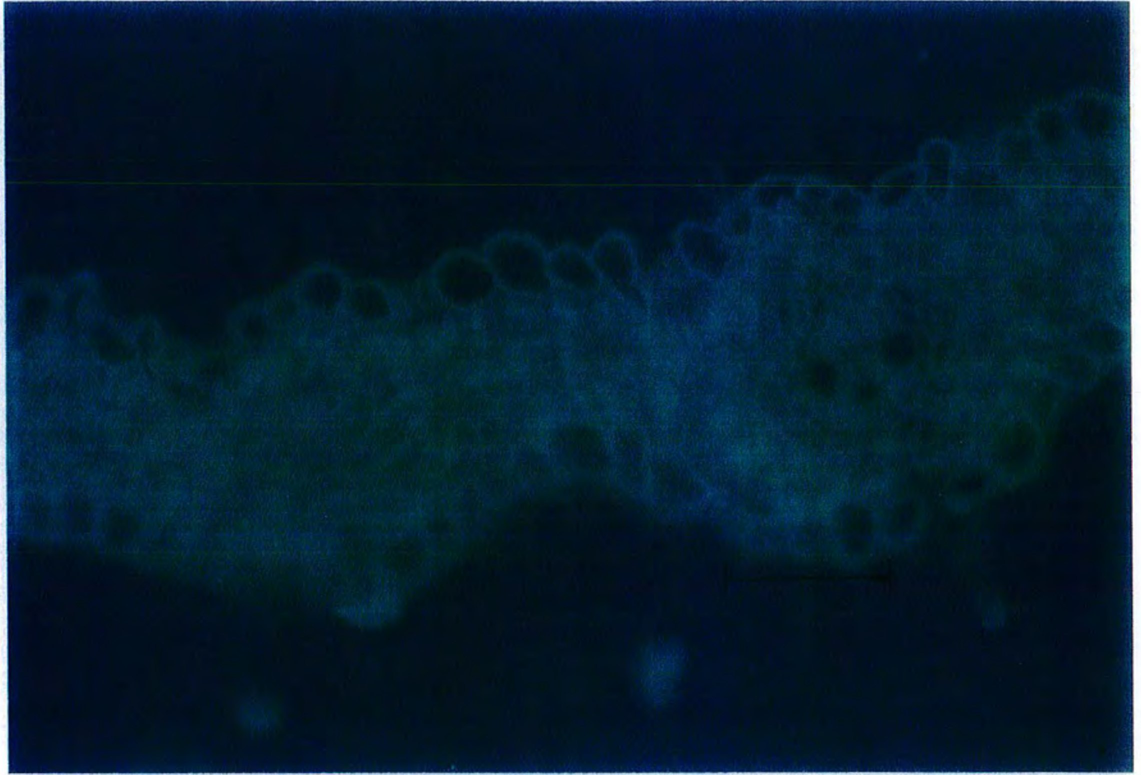


Plate 10

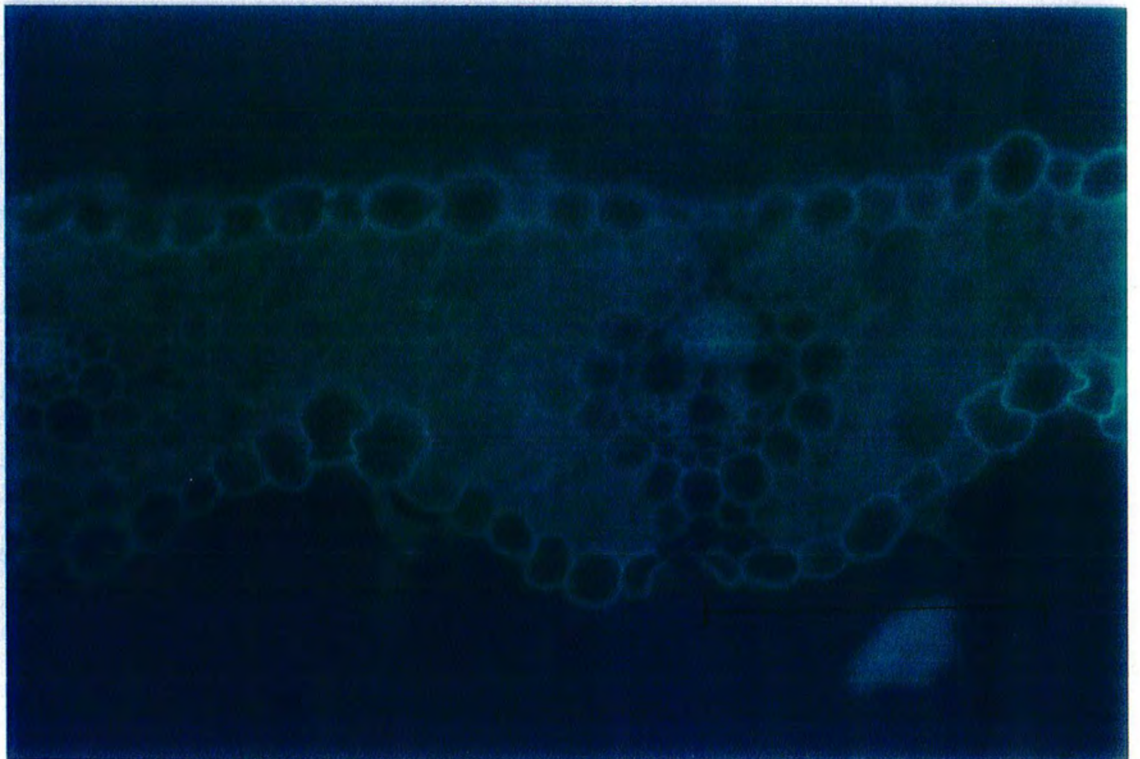


Plate 11

Transverse section from the tip of the control GEN1 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m.

Plate 12

Transverse section from the tip of the GEN2 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m.

Plate 11

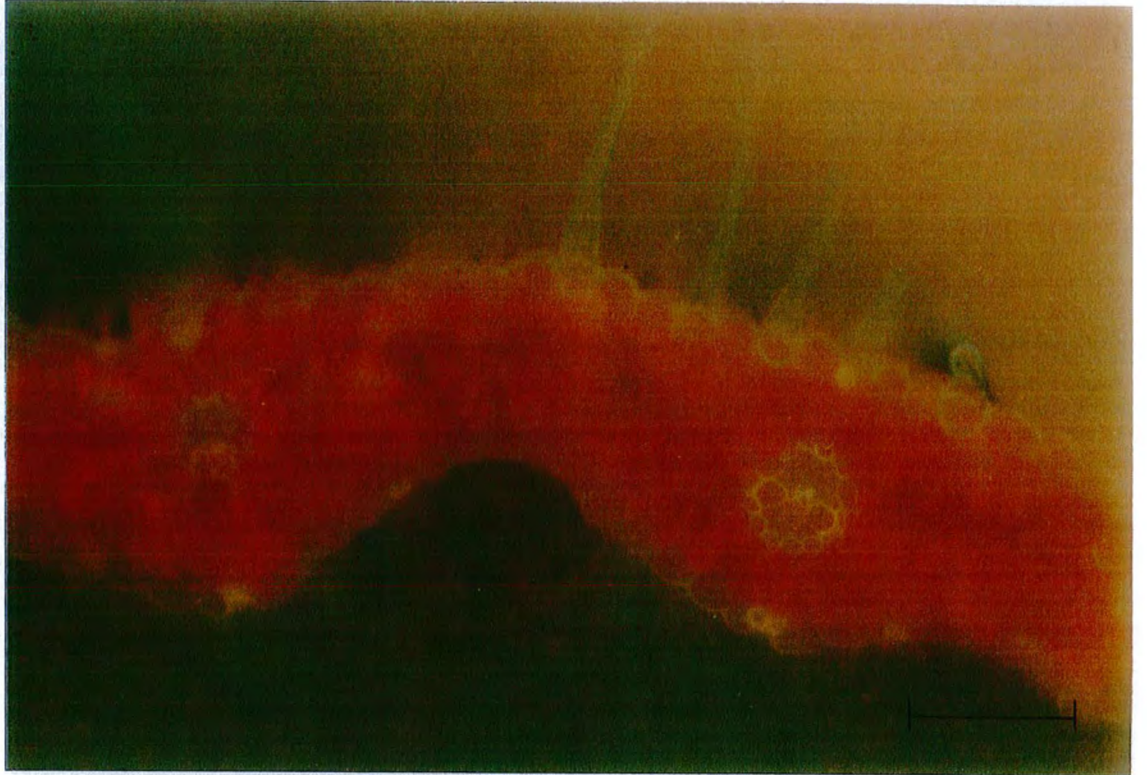


Plate 12

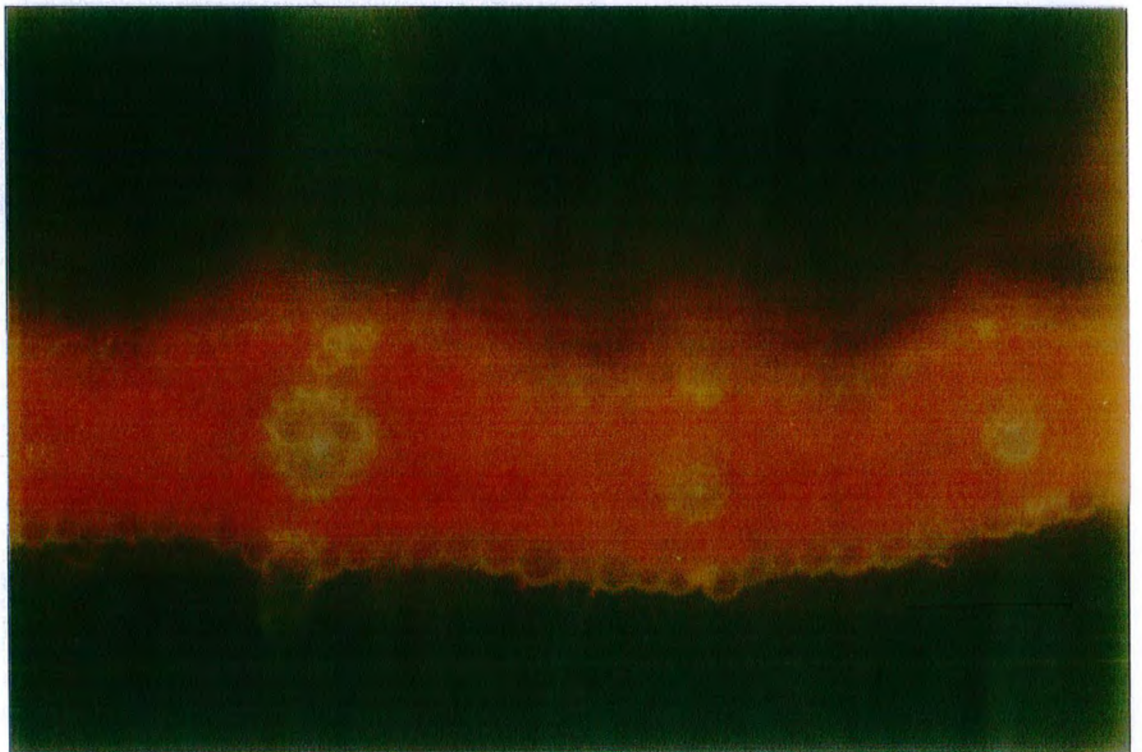


Plate 13

Transverse section from the middle of the control GEN1 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 14

Transverse section from the middle of the GEN2 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 13

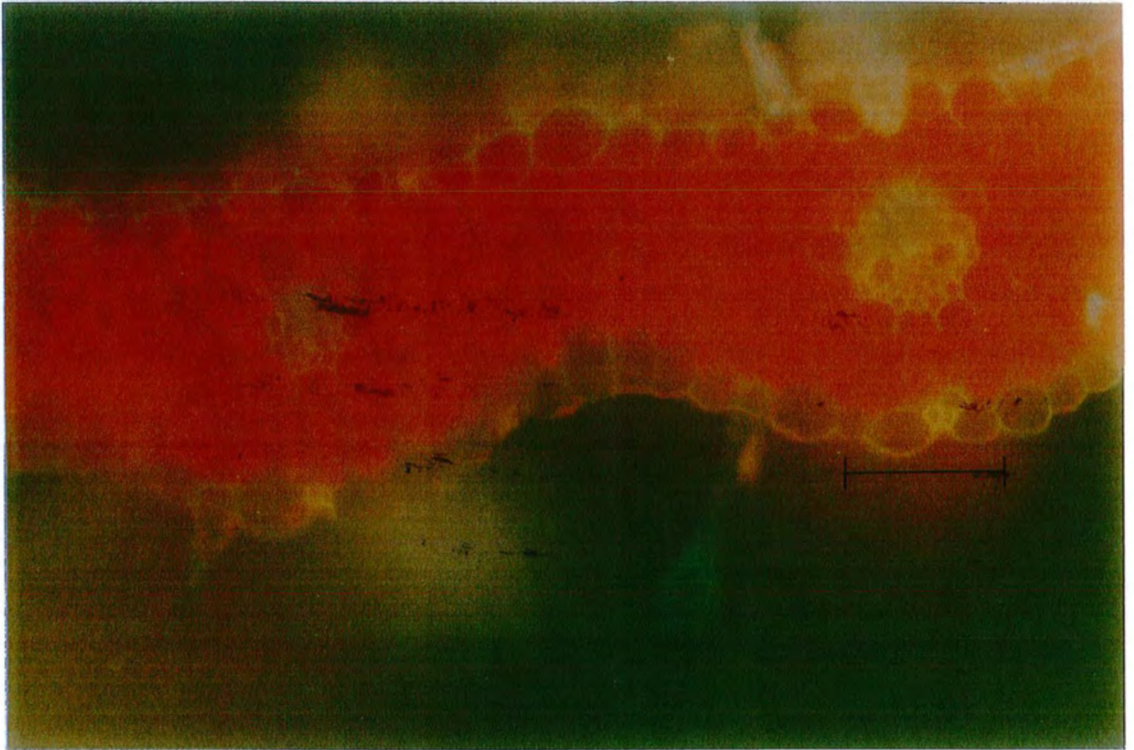


Plate 14

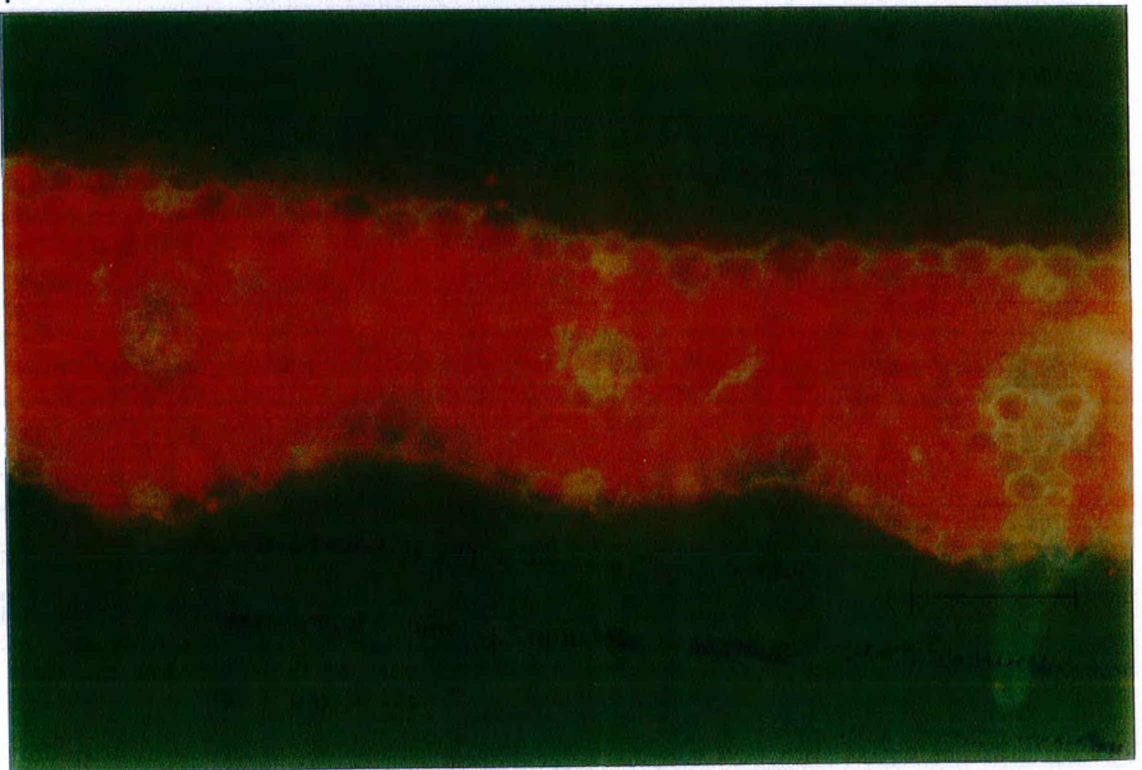


Plate 15

Transverse section from the base of the control GEN1 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 16

Transverse section from the base of the GEN2 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 15

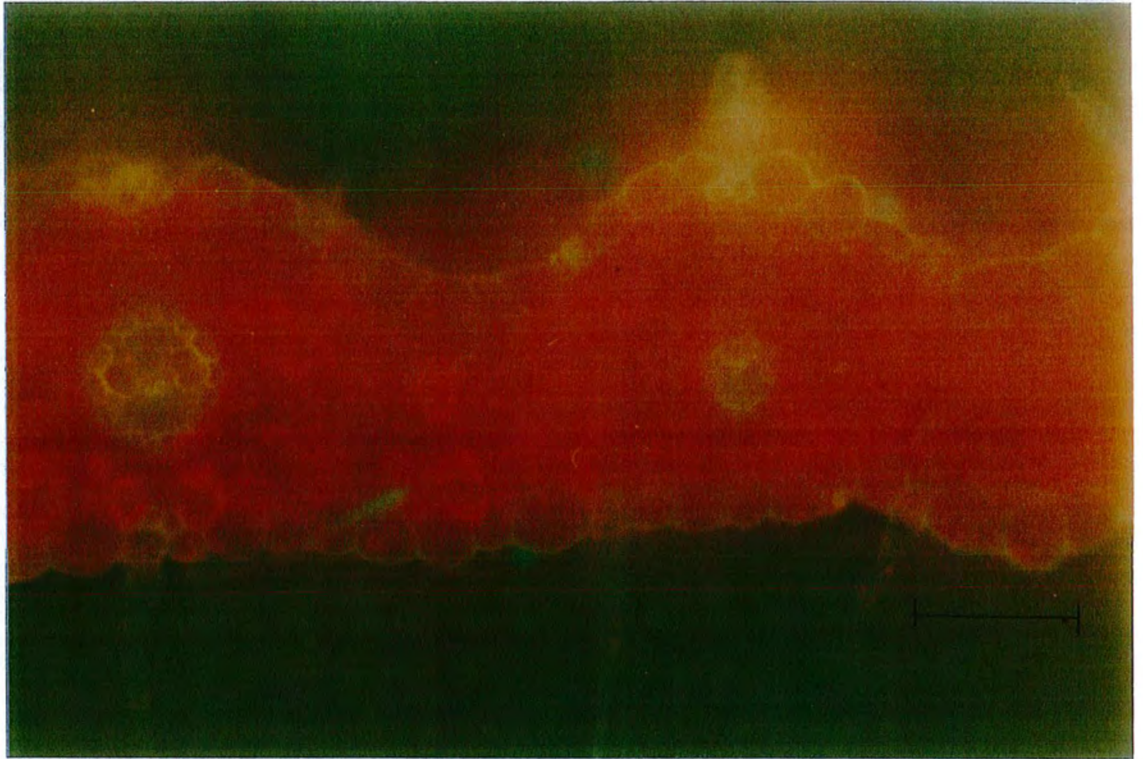
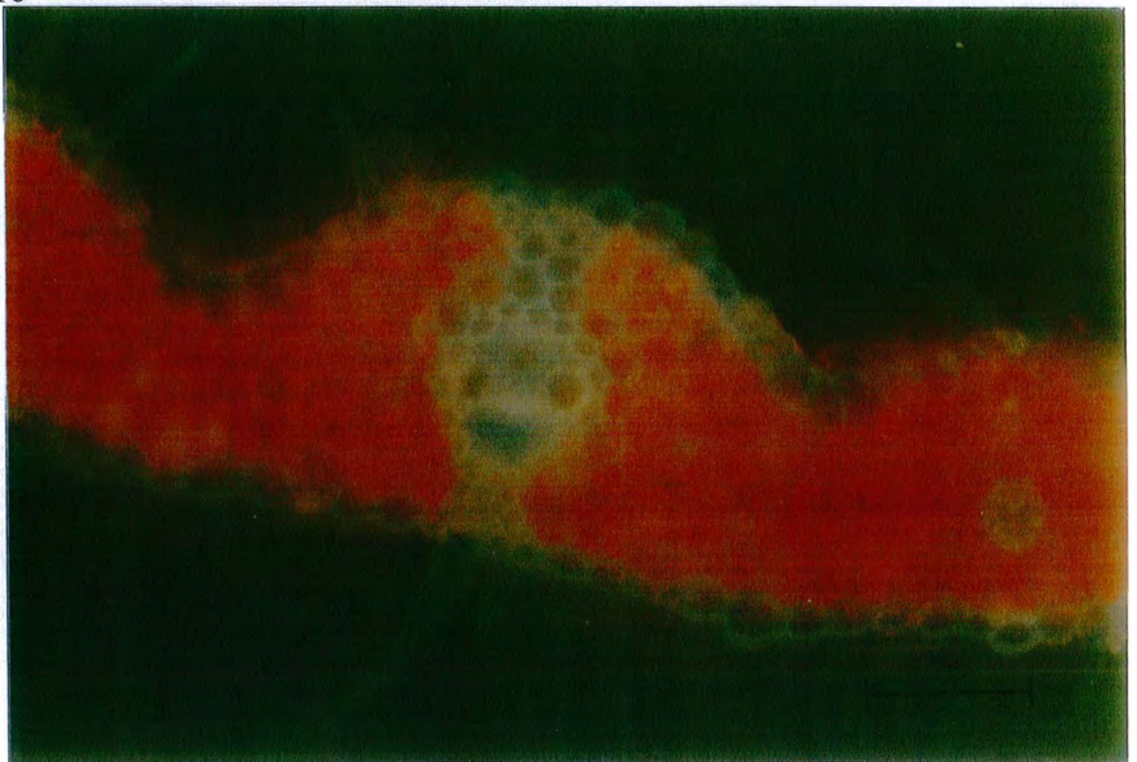


Plate 16



segments. Similarly, under the 7-day watering regime, the leaf sections (tip, mid, and base) of the GEN2 plants again showed no difference in chlorophyll presence when compared with the counterpart of the GEN1 control plants (Plates 17, 18, 19, 20, 21, and 22). However, one notable difference between the GEN1 and GEN2 plants was the enhanced blue fluorescent deposition seen in the upper and lower epidermal cells of the mid and base section of the GEN2 leaves (Plates 20 and 22). This fluorescence was also more pronounced in the GEN1 7-day watered plants compared with the 2-day watered plants. This indicates that there were changes in the retaining patterns of the cell walls related to watering regimes which appear to be enhanced by Cycocel pre-treatment.

From an analysis of the fluorescent stain patterns of the leaf sections it appears that there was no consistent selective deposition of stain into the walls of the middle and basal segments in either GEN1 or GEN2 plants under both 2 and 7-day watering regimes (Plates 25, 26, 27, 28, 31, 32, 33, and 34). However, a slight difference in staining was observed in tip sections where greater deposition was seen in the epidermis of GEN2 plant material under both watering regimes (Plates 23, 24, 29, and 30). This difference may be related to reduced water loss from the tip sections of the GEN2 leaves.

A further anatomical investigation was initiated for the stressed GEN1 and GEN2 wheat plants. The water supply of these plants was ceased after the 4th leaf reached maturity. In the GEN1 plants water was withheld for 10 days after the plants had been grown under a 2-day watering regime. GEN1 plants subjected to a 7-day watering regime were dehydrated and not suitable for analysis after 10 days. GEN2 plants used in this study were from specimens subjected to 15 days without water at the end of a 7-day water regime. Under these relatively sever conditions the GEN2 plants continued to be turgid and were suitable for analysis.

Plate 17

Transverse section from the tip of the control GEN1 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 18

Transverse section from the tip of the GEN2 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 17

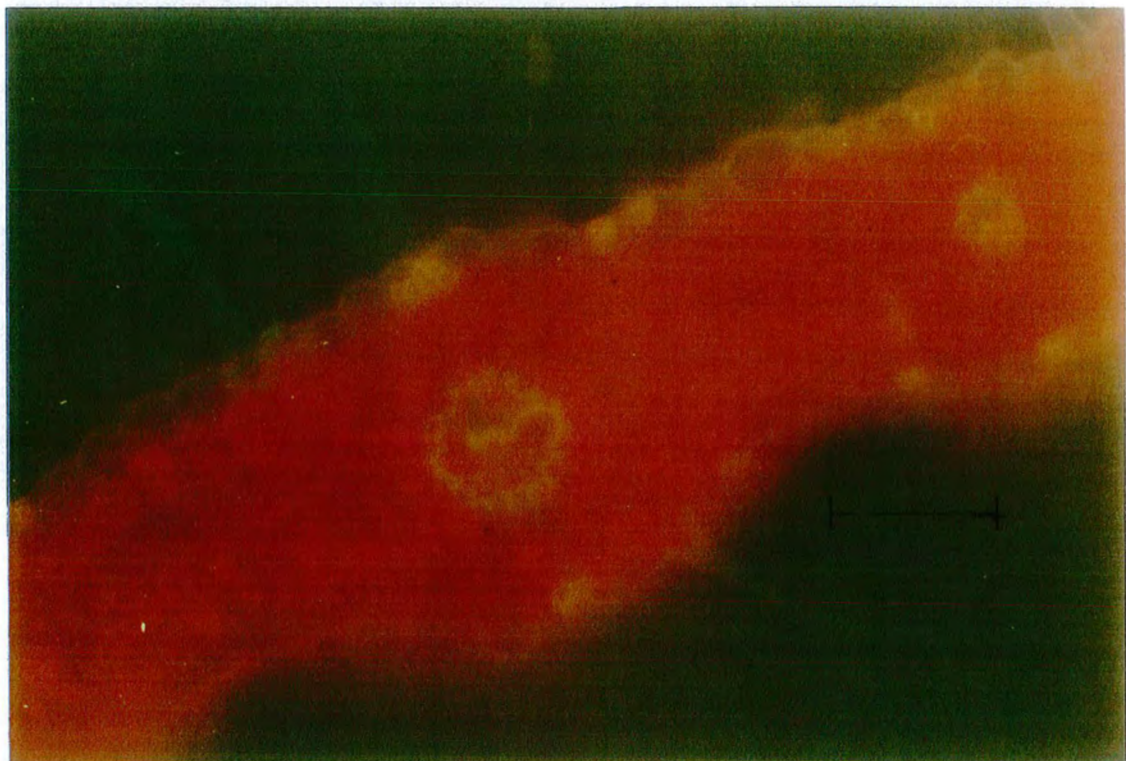


Plate 18

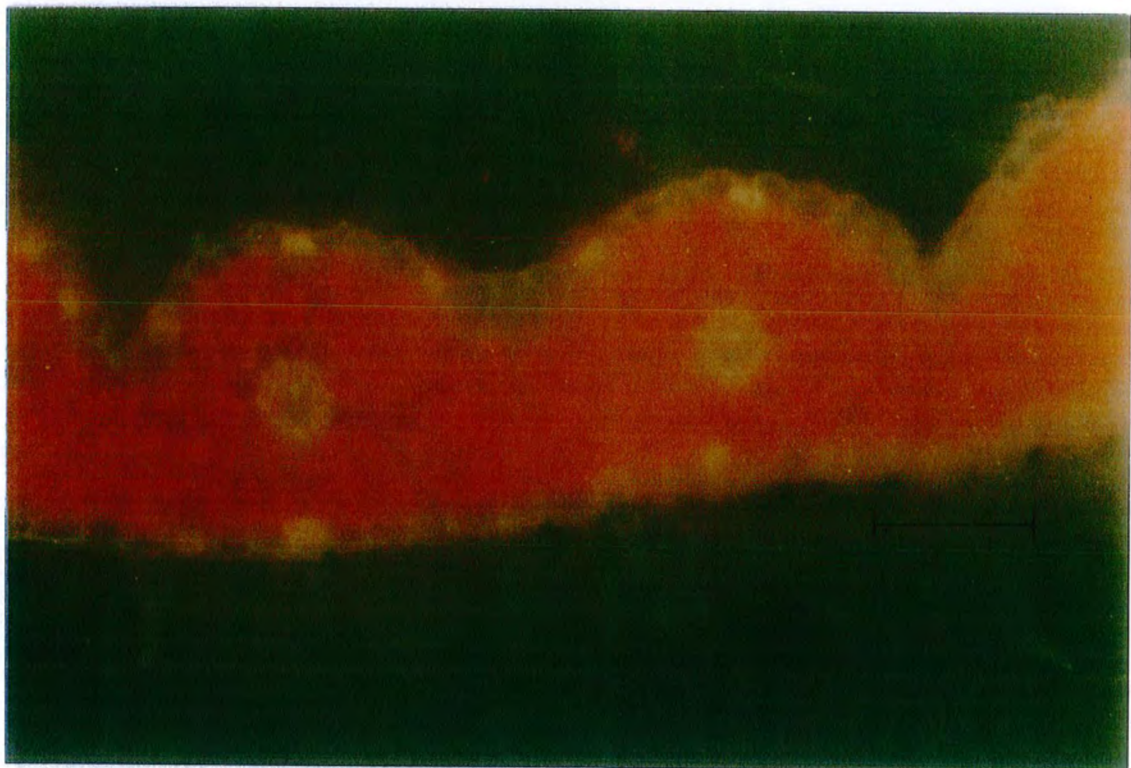


Plate 19

Transverse section from the middle of the control GEN1 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance. Scale bar = 100 *um*.

Plate 20

Transverse section from the middle of the GEN2 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance. Scale bar = 100 *um*.

Plate 19

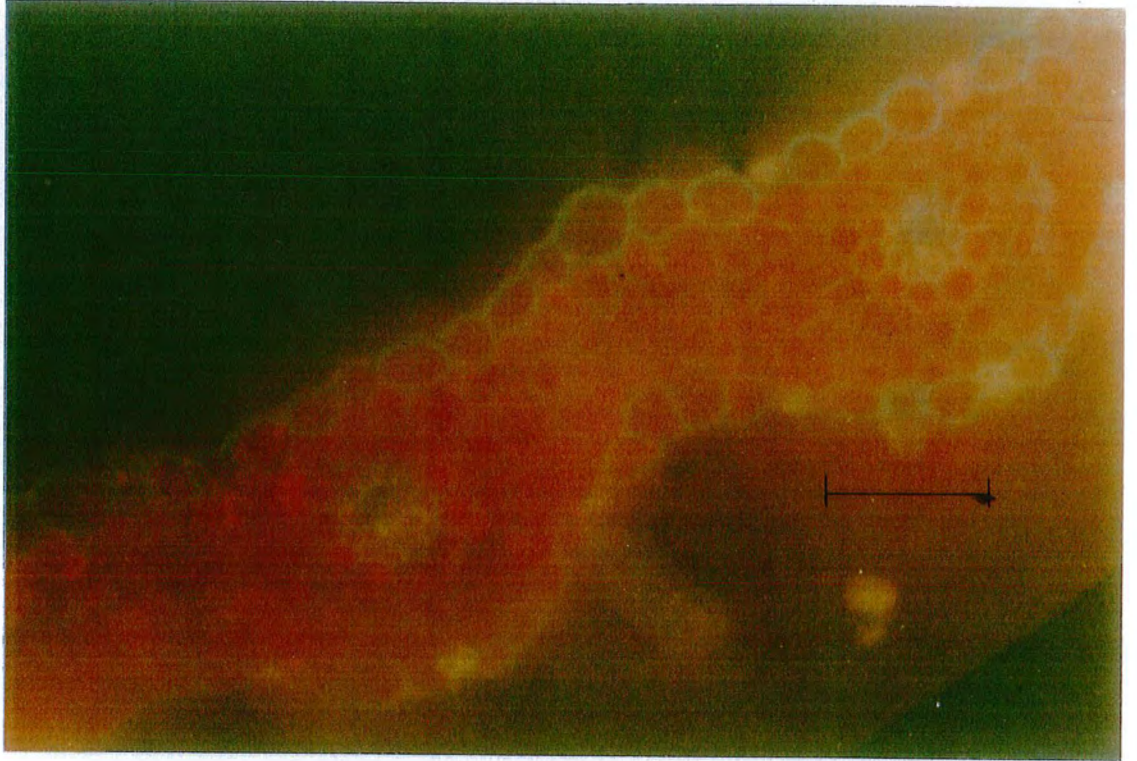


Plate 20

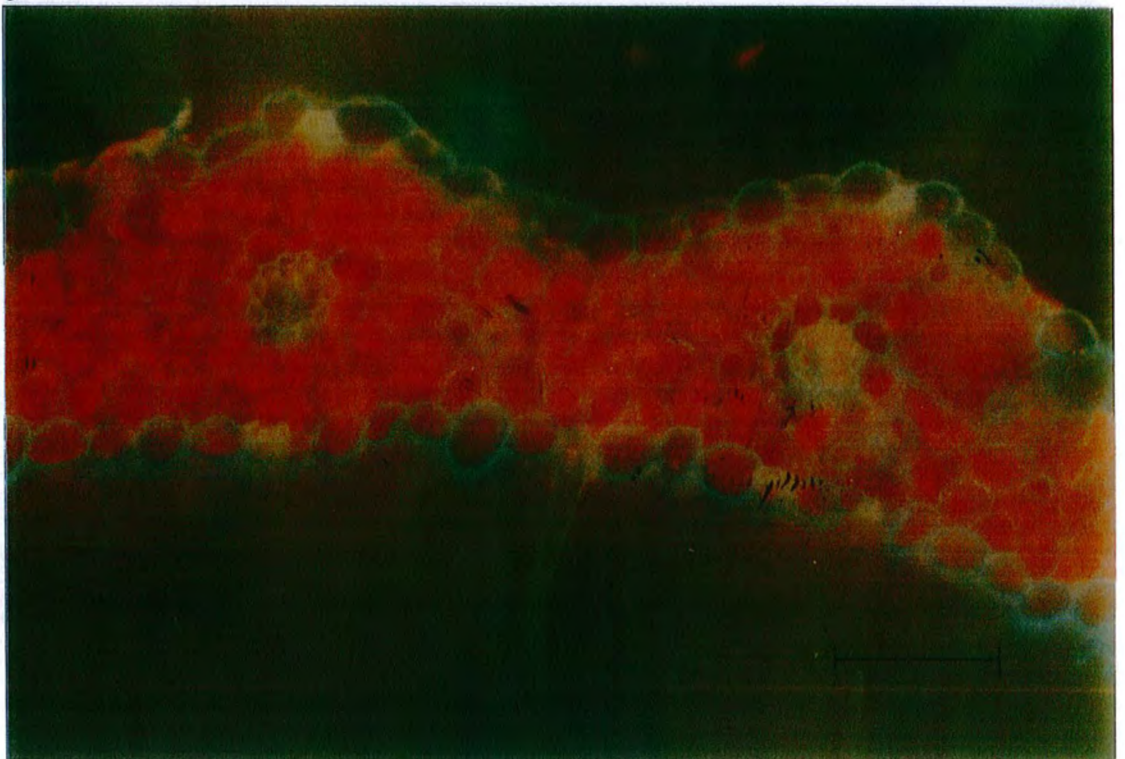


Plate 21

Transverse section from the base of the control GEN1 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 22

Transverse section from the base of the GEN2 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter to enhance chlorophyll. Scale bar = 100 *um*.

Plate 21

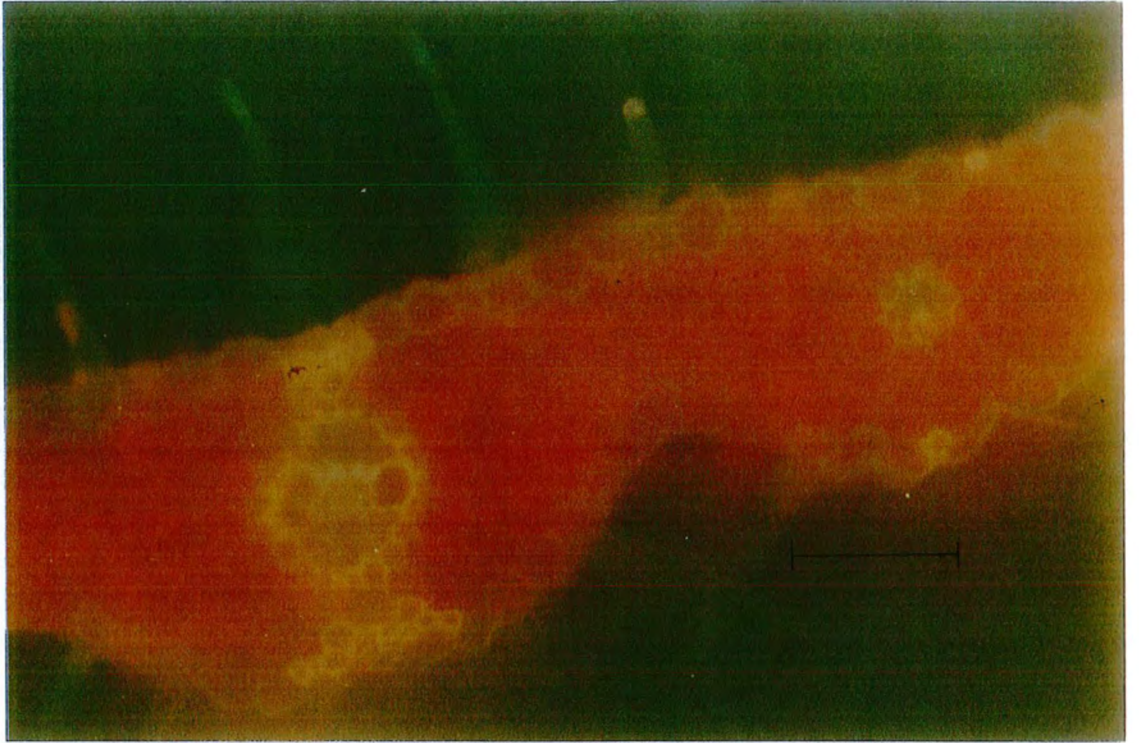


Plate 22

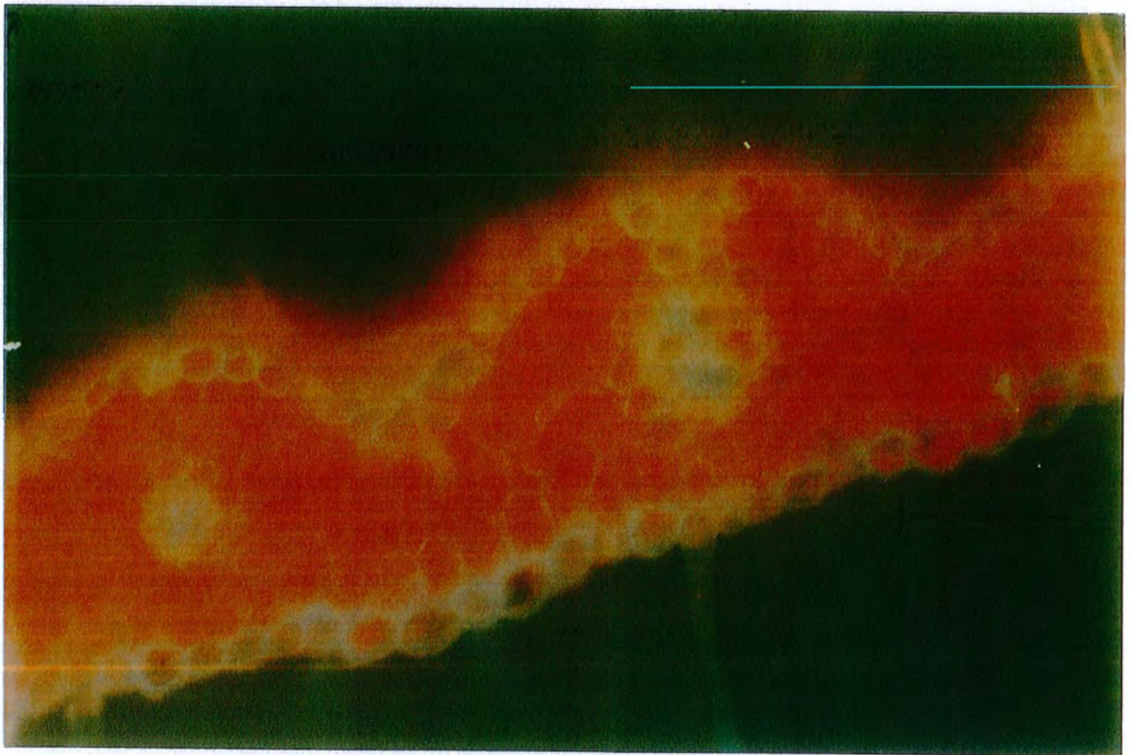


Plate 23

Transverse section from the tip of the control GEN1 wheat leaf, sampled from the 2-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *um*.

Plate 24

Transverse section from the tip of the GEN2 wheat leaf, sampled from the 2-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *um*.

Plate 23

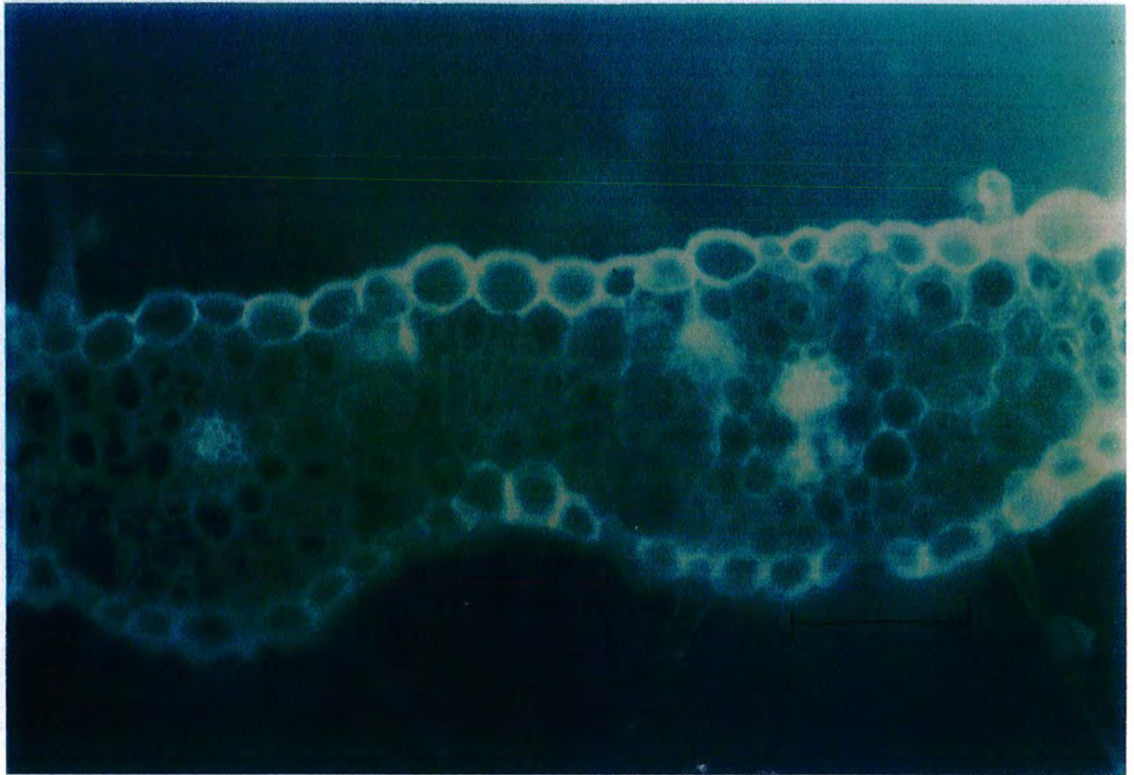


Plate 24

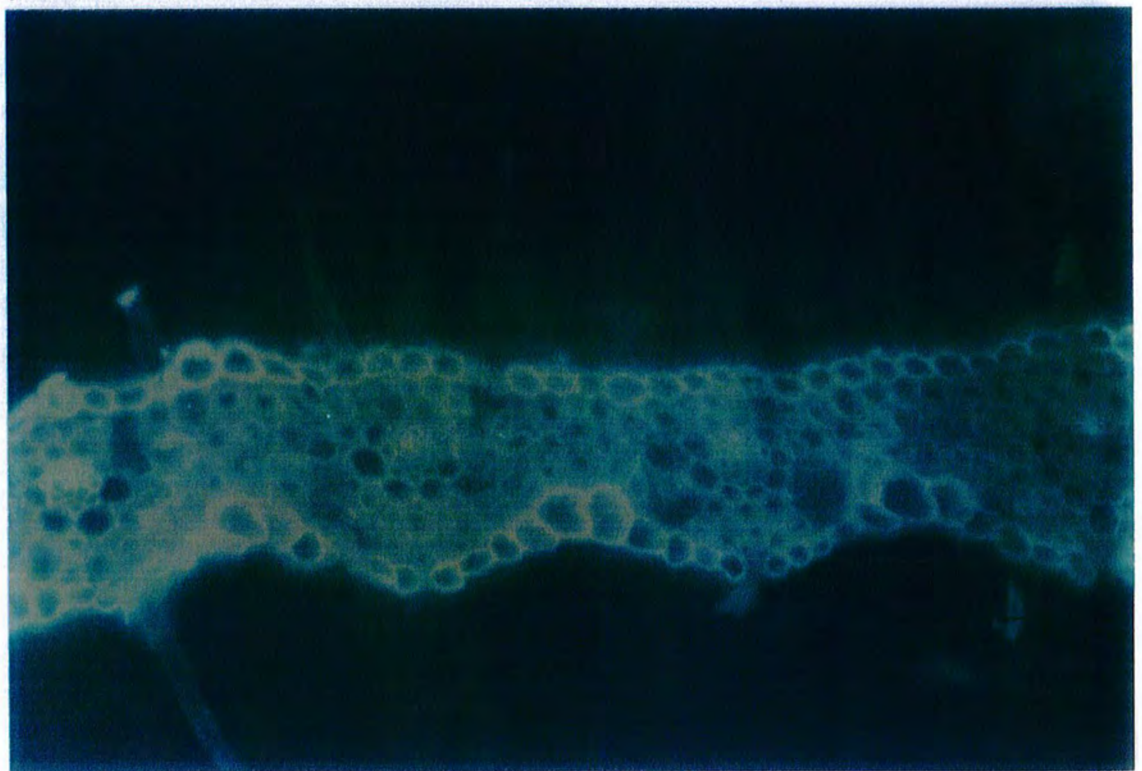


Plate 25

Transverse section from the middle of the control GEN1 wheat leaf, sampled from the 2-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *um*.

Plate 26

Transverse section from the middle of the GEN2 wheat leaf, sampled from the 2-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *um*.

Plate 25

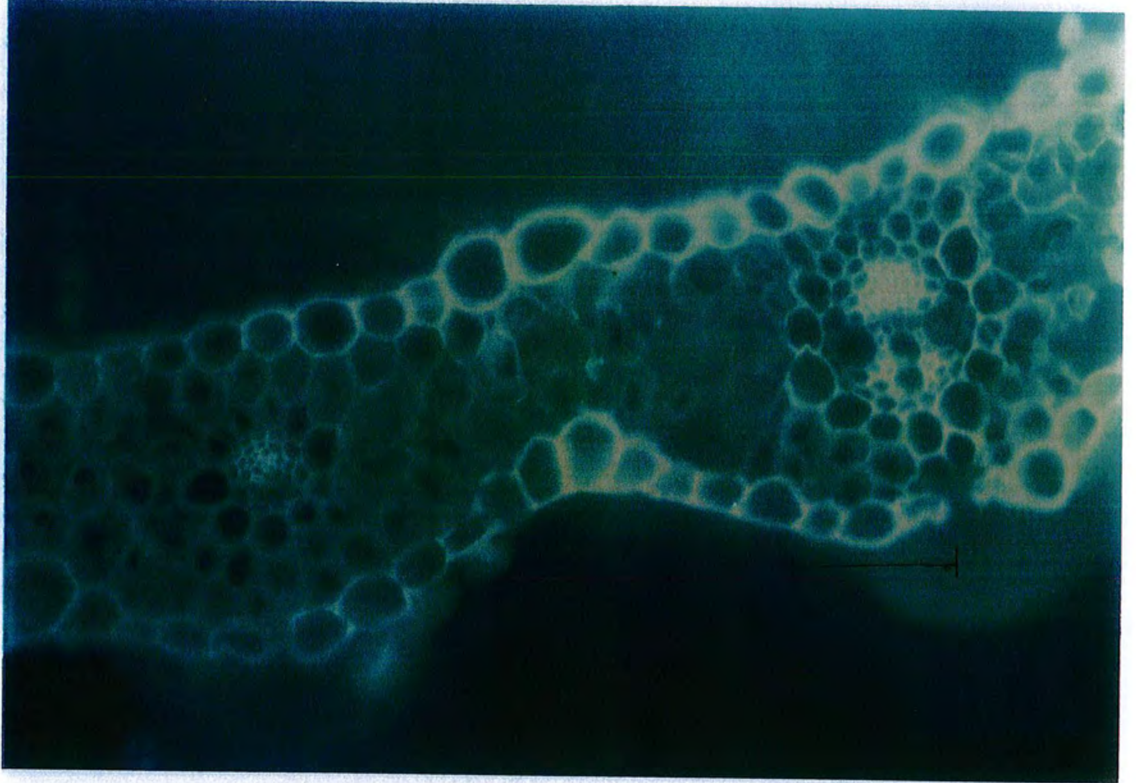


Plate 26

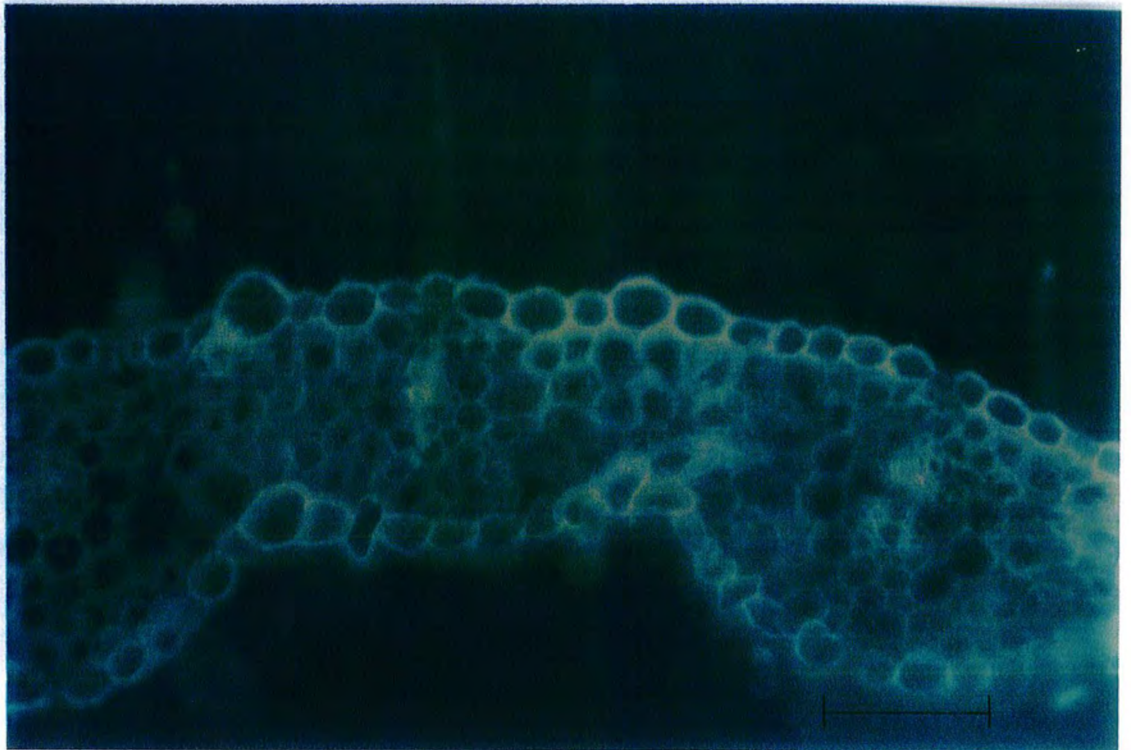


Plate 27

Transverse section from the base of the control GEN1 wheat leaf, sampled from the 2-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *um*.

Plate 28

Transverse section from the base of the GEN2 wheat leaf, sampled from the 2-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *um*.

Plate 27

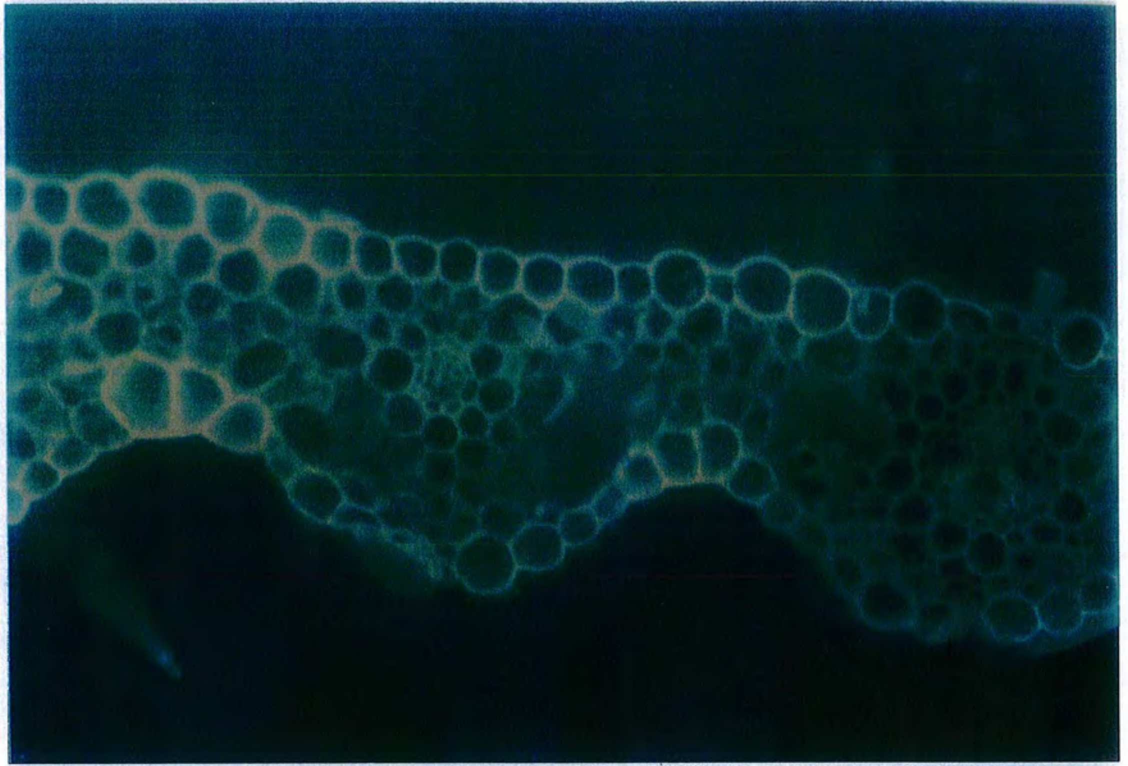


Plate 28

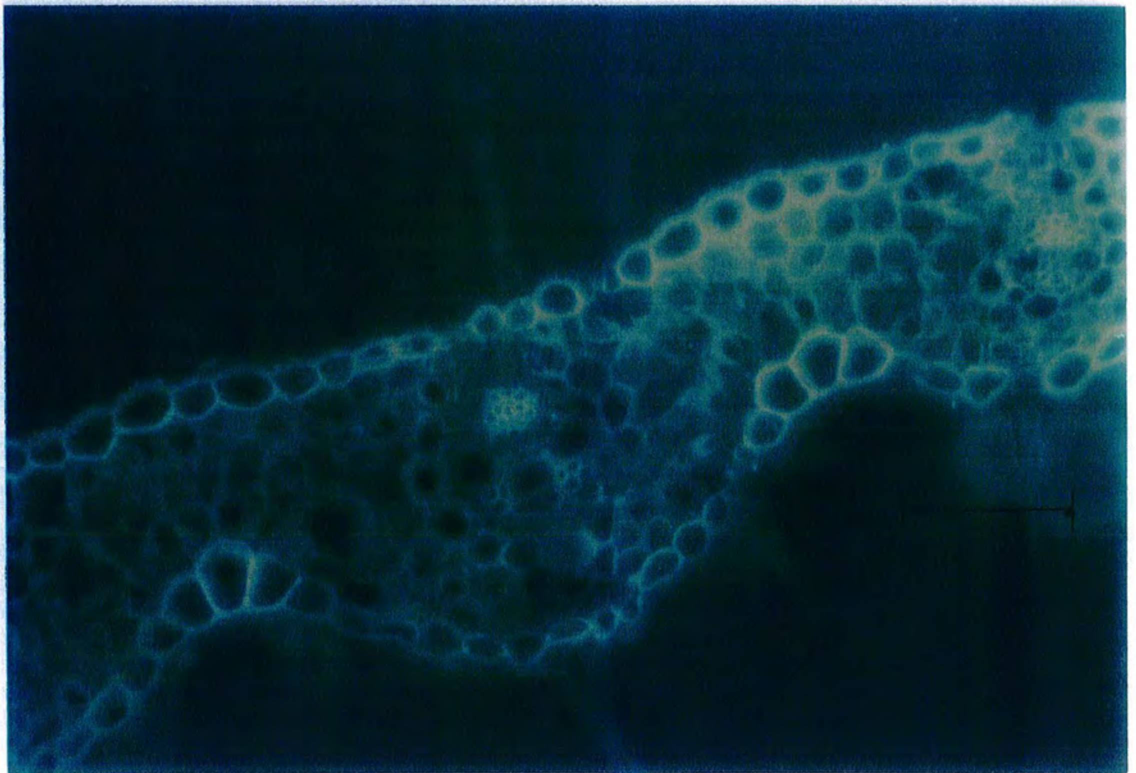


Plate 29

Transverse section from the tip of the control GEN1 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m.

Plate 30

Transverse section from the tip of the GEN2 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 29

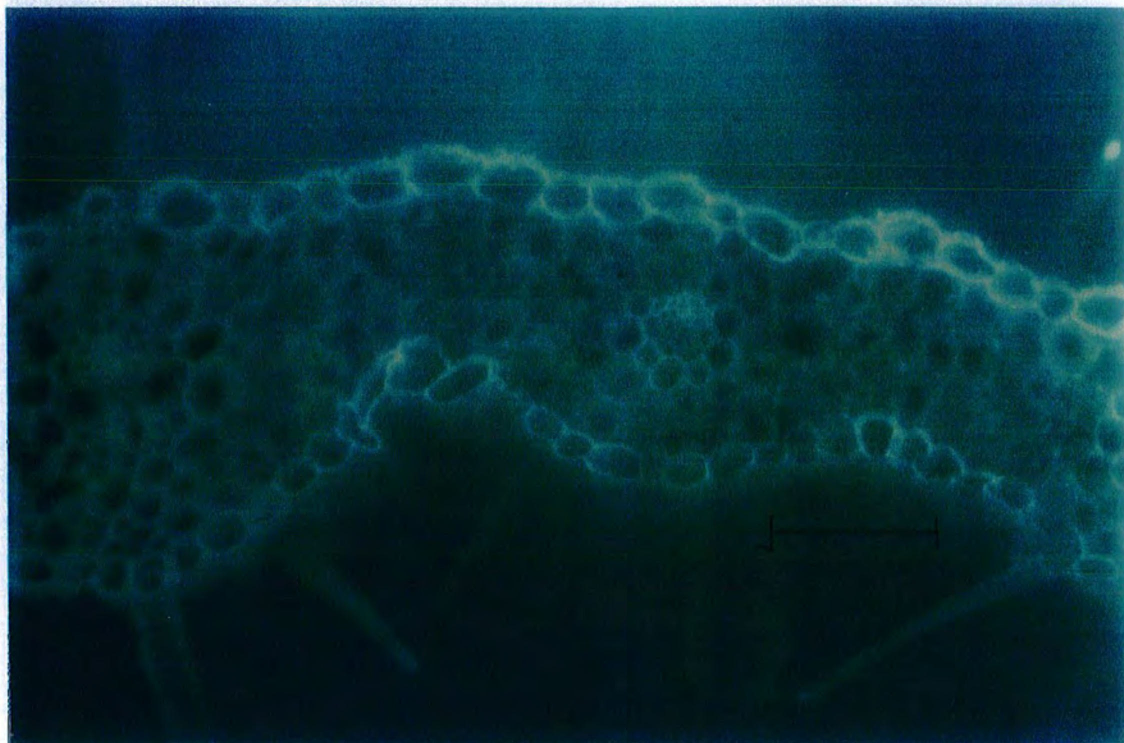


Plate 30

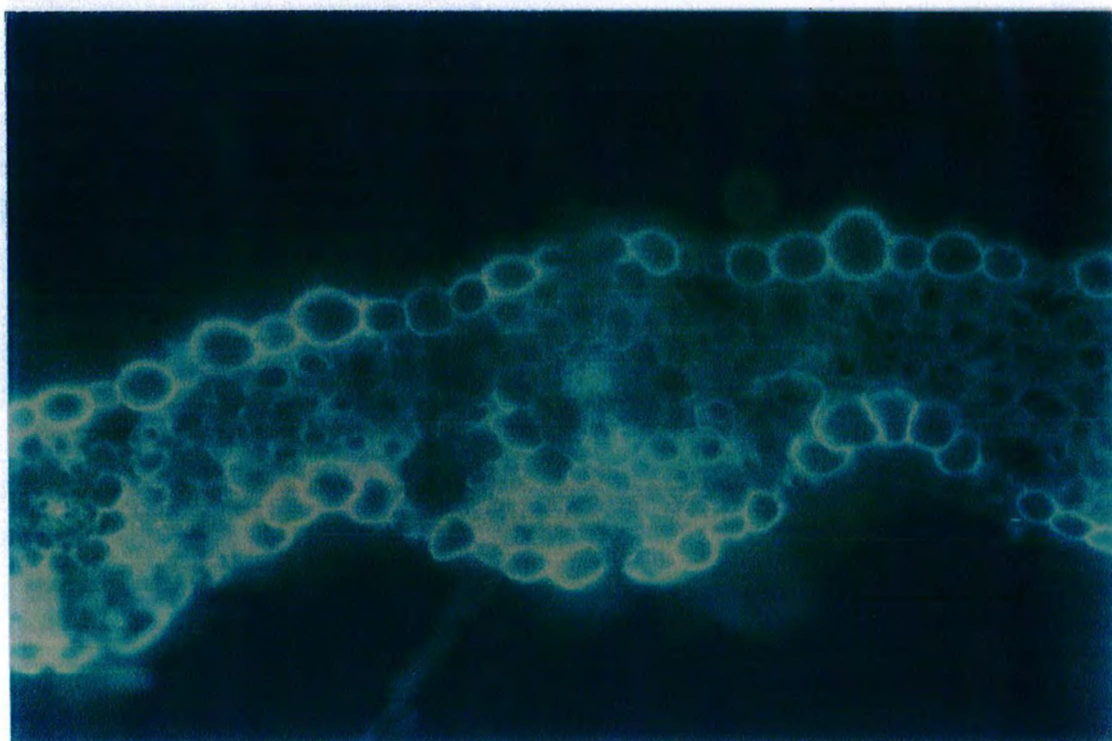


Plate 31

Transverse section from the middle of the control GEN1 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 32

Transverse section from the middle of the GEN2 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 31

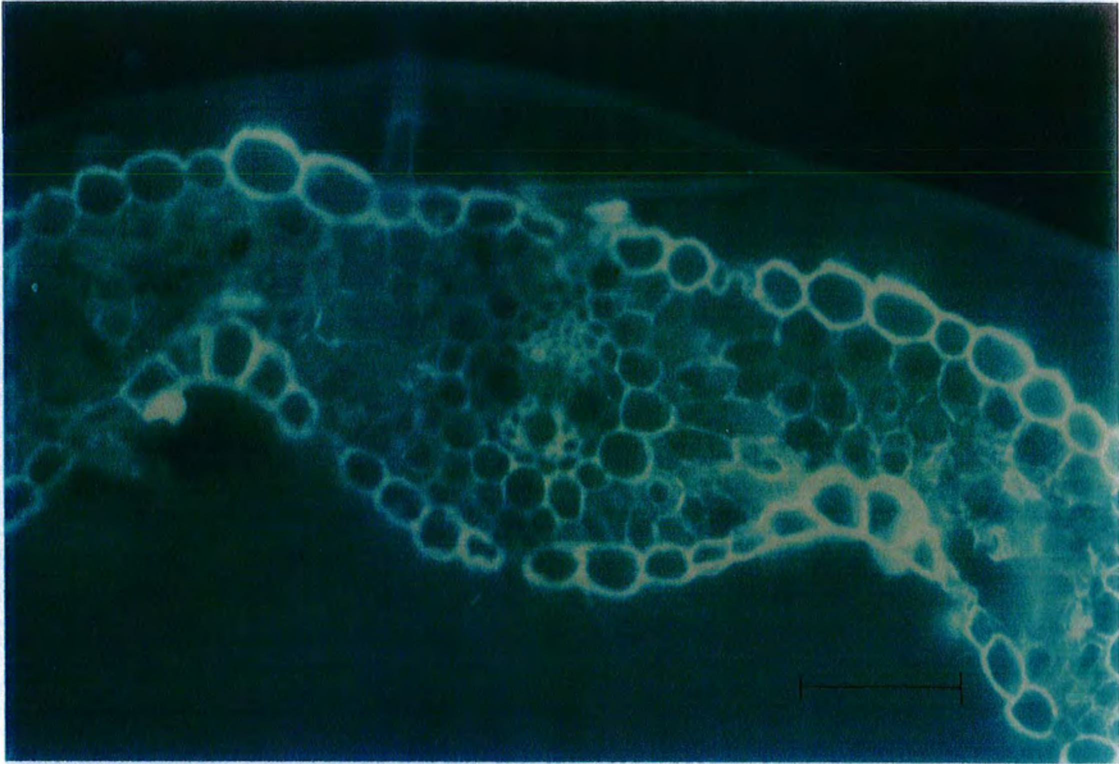


Plate 32

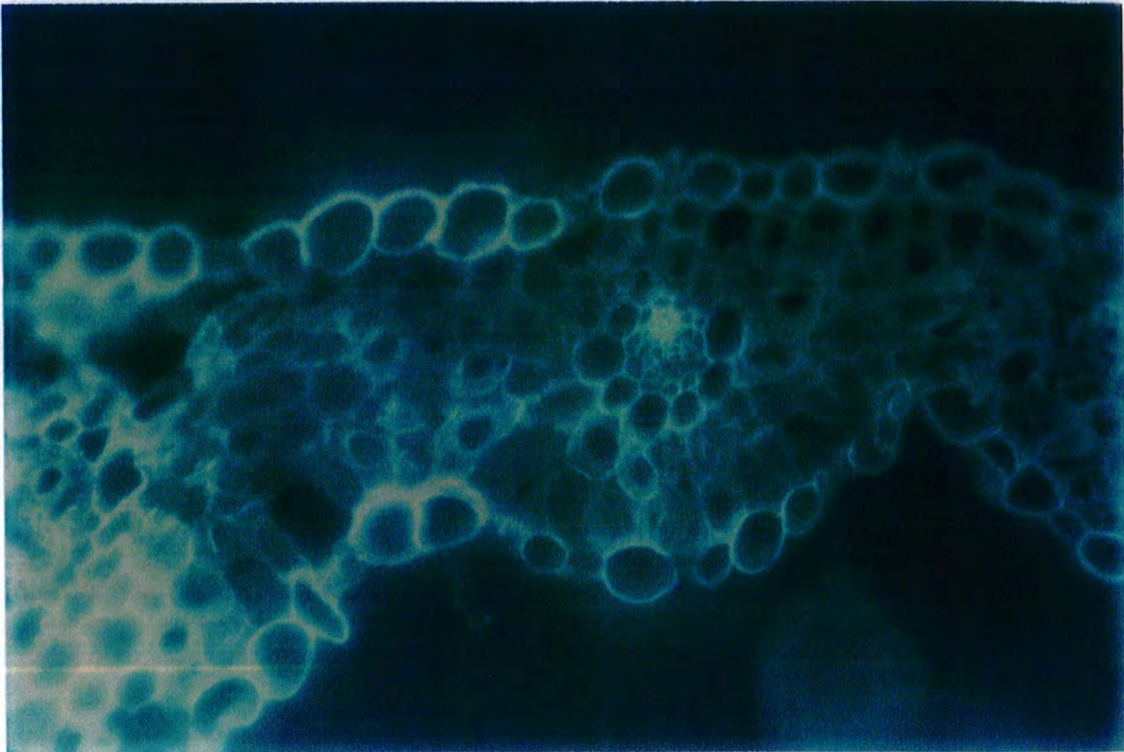


Plate 33

Transverse section from the base of the control GEN1 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 34

Transverse section from the base of the GEN2 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 33

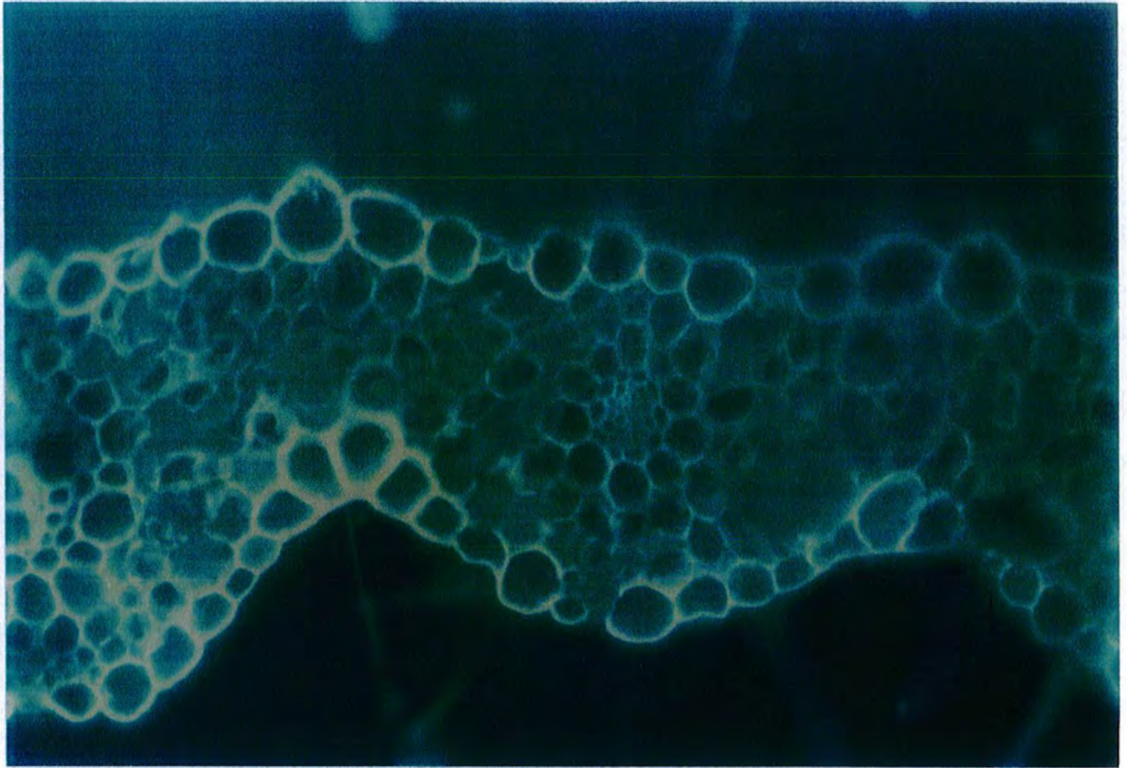
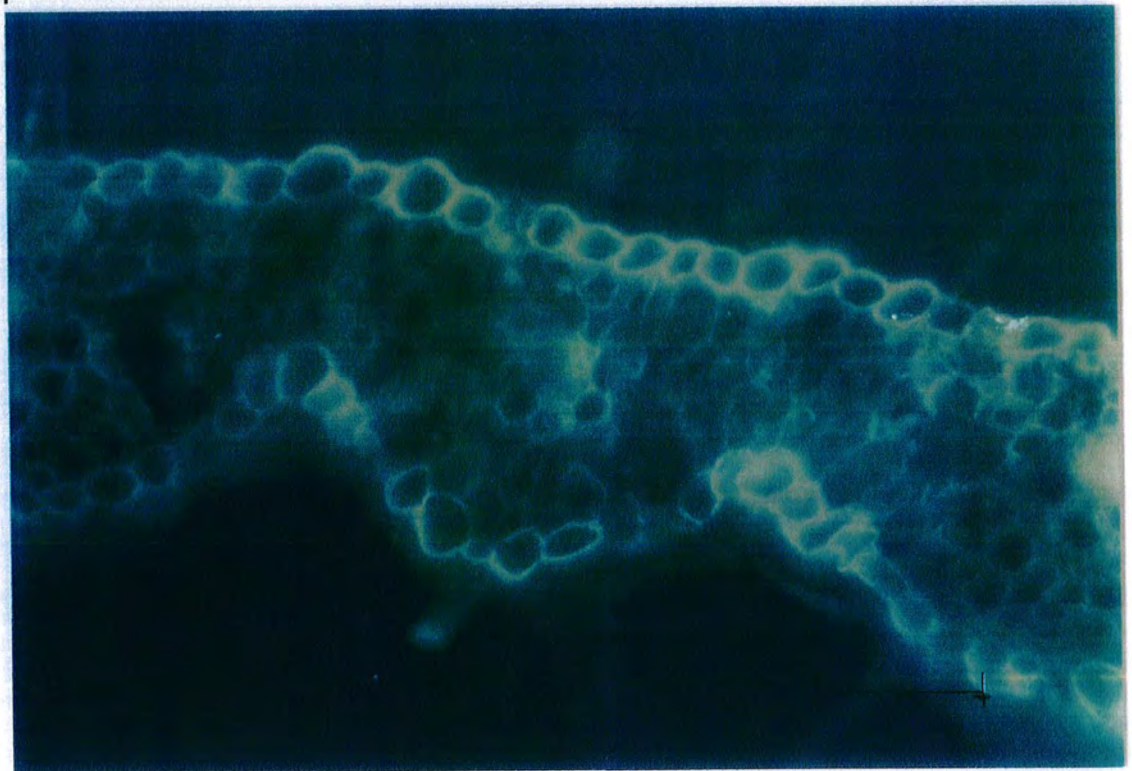


Plate 34



Plates 35 and 36 show typical chlorophyll illumination patterns of the GEN1 and GEN2 mid leaf sections respectively. From Plate 36 it can be seen that the chlorophyll fluorescence was greater in the GEN2 plants than GEN1. The GEN2 plants clearly showed an ability to withstand stress in term of chlorophyll content.

As was noted in the earlier reported analyses of tip, middle, and basal segments there was no consistent dye fluorescence of the epidermis after treatment with calcofluor for the GEN1 and GEN2 plants (Plates 37 and 38).

3.4.2 Root tissue

Hydroponic culture techniques allowed further analyses of the anatomy of the roots. This work was performed on GEN1 tissue only from derived from Cycocel pre-treated caryopses and control plants. The combination of microscopic techniques and the fluorescent dyes allowed to further investigation any anatomical changes associated with Cycocel pre-treatment. In this part of the study, the roots of GEN1 plants grown in hydroponics (NS or NS + PEG) were sectioned from the mature top part of the root then examined under the fluorescent microscope with an AO fluorescent dye, as described in the Methods.

The cell cortex of the Cycocel pre-treated GEN1 roots, grown in NS conditions, showed a dense bright fluoresced deposition when compared to the control GEN1 roots (Plate 39 and 40). However, the control plants exposed to the same conditions did show a slight scattered deposition in the cortex. This deposition into the cell walls appeared to represent a response to Cycocel treatment of roots. When plants grown in NS + PEG this difference was much more apparent (Plates 41 and 42). There was bright fluorescence in the cortex of the Cycocel treated plants with only slight fluorescent in the control.

Plate 35

Transverse section of the stressed (10 days water cessation) control GEN1 wheat leaf, sampled from the 2-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m

Plate 36

Transverse section of the stressed (15 days water cessation) GEN2 wheat leaf, sampled from the 7-day watering regime. Unstained specimens were mounted in water, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m

Plate 35

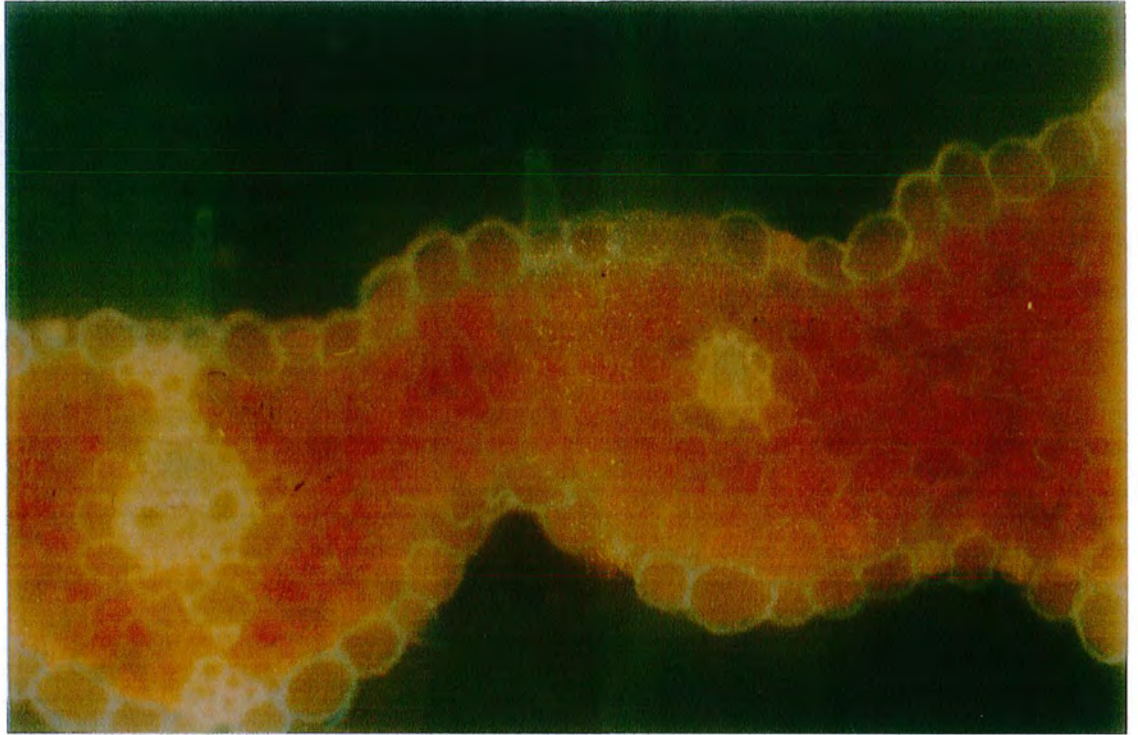


Plate 36

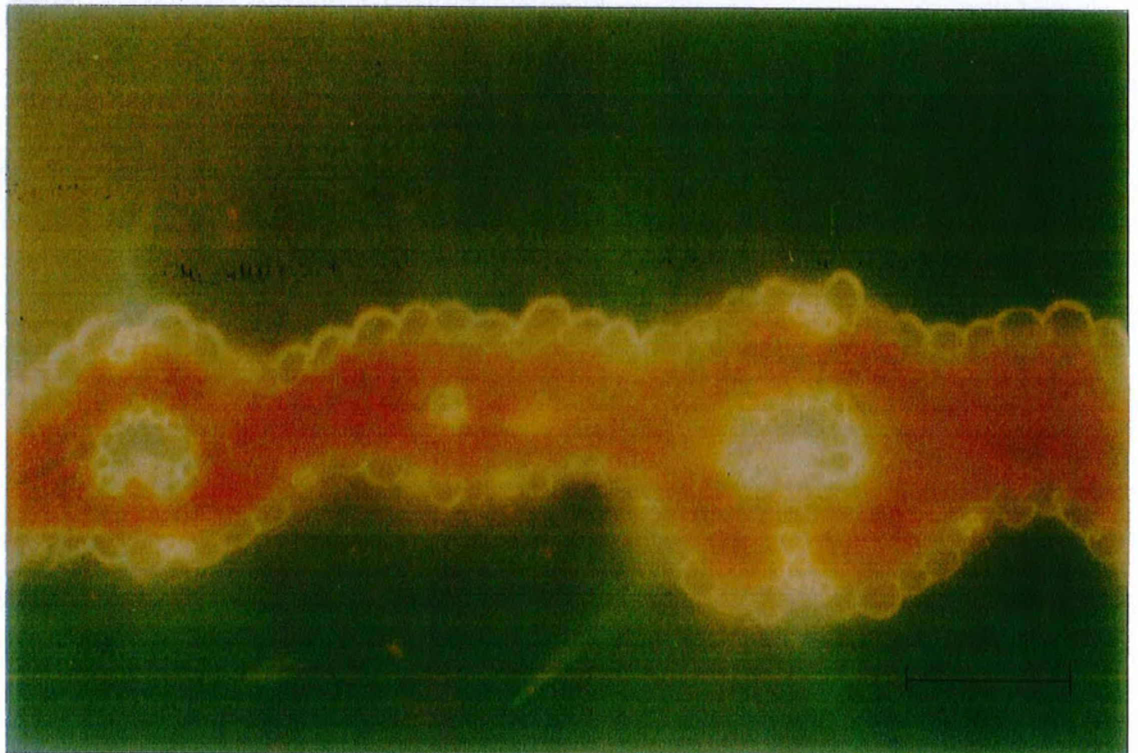


Plate 37

Transverse section of the stressed (10 days water cessation) control GEN1 wheat leaf, sampled from the 2-day watering regime, . Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 38

Transverse section of the stressed (15 days water cessation) GEN2 wheat leaf, sampled from the 7-day watering regime. Specimens were stained with Calcofluor, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with an ultraviolet filter. Scale bar = 100 *u*m

Plate 37

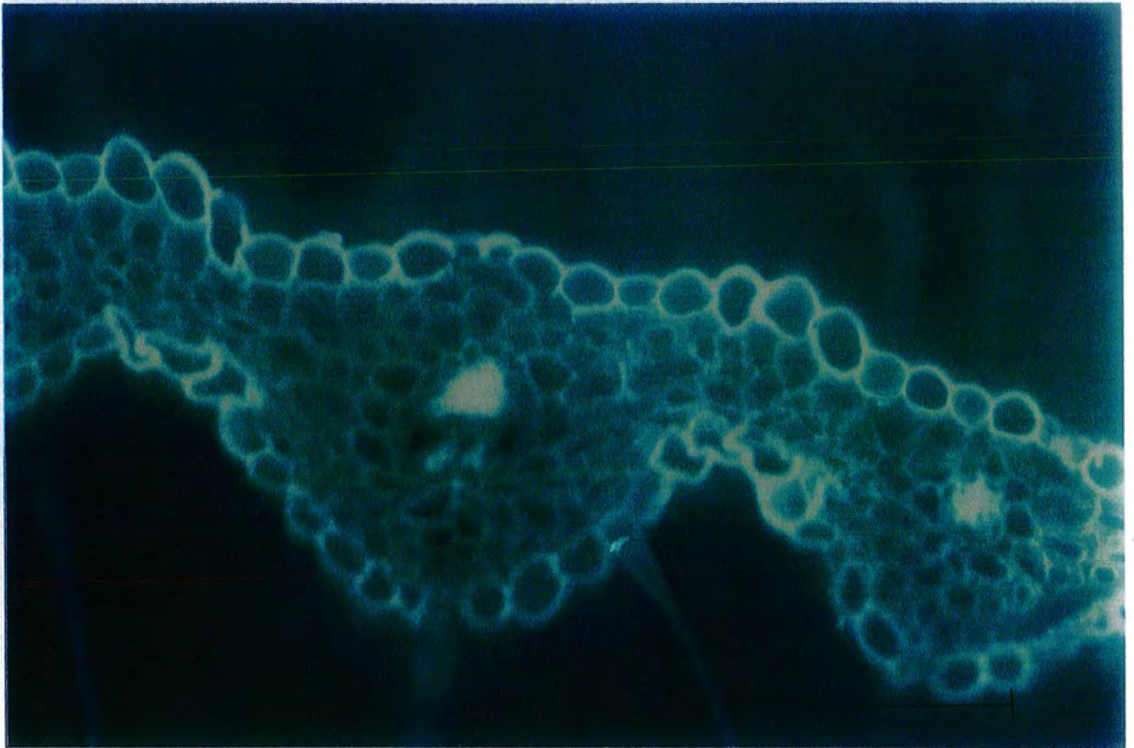


Plate 38

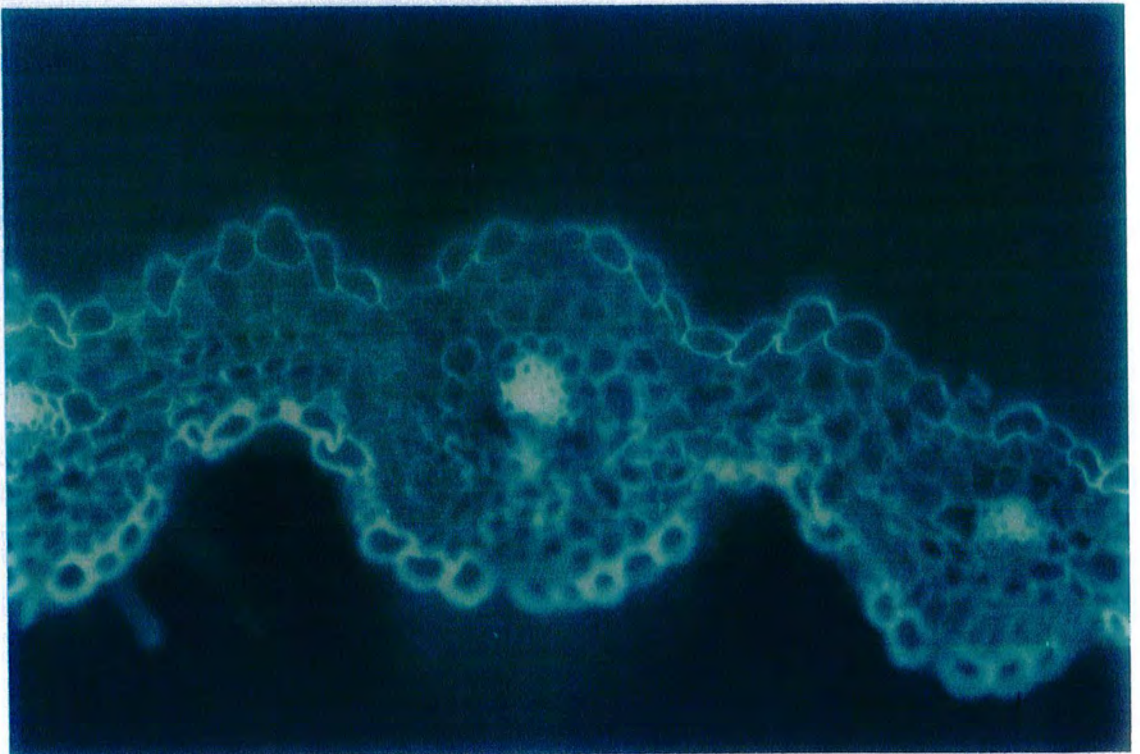


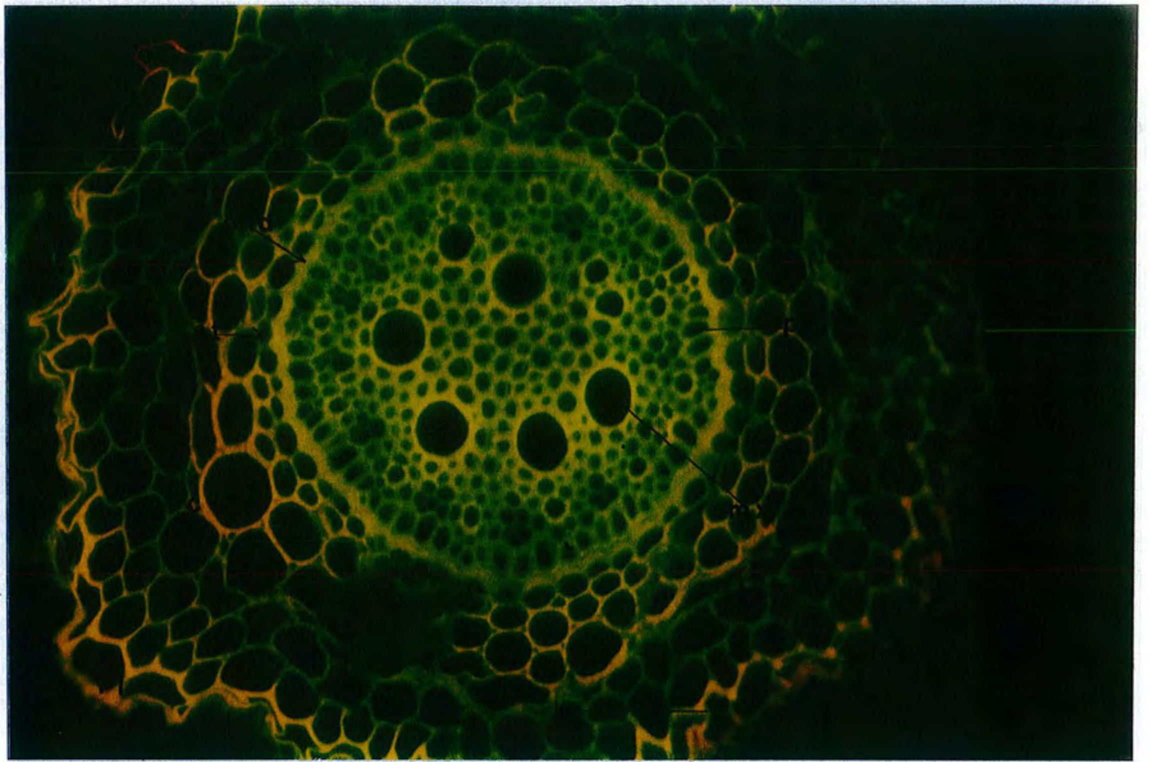
Plate 39

Section of root of the Cycocel pre-treated GEN1 plant, illustrating the root cortex, sampled from the hydroponic NS solution alone. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Abbreviations: c = cortex; pl = piliferous layer; st = stele; end = endodermis; p = pericycle; mx = metaxylem. Scale bar = 100 *u*m.

Plate 40

Section of root of the control (none treated) GEN1 plant, illustrating the root cortex, sampled from the hydroponic NS solution alone. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *u*m.

Plate 39



Cell cortex

Plate 40

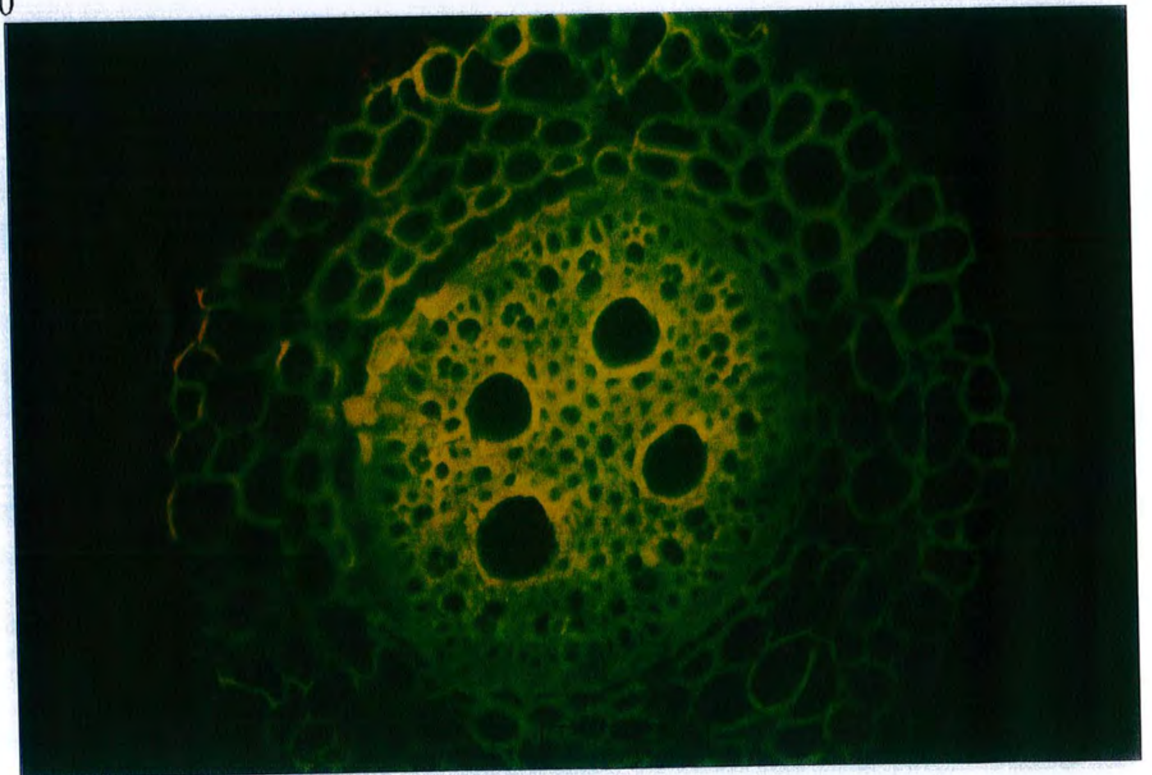


Plate 41

Section of root of the Cycocel pre-treated GEN1 plants, illustrating the root cortex, sampled from the hydroponic NS + PEG solution. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a violet filter. Scale bar = 100 *um*.

Plate 42

Section of root of the control (none treated) GEN1 plants, illustrating the root cortex, sampled from the hydroponic NS + PEG solution. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a violet filter. Scale bar = 100 *um*.

Plate 41

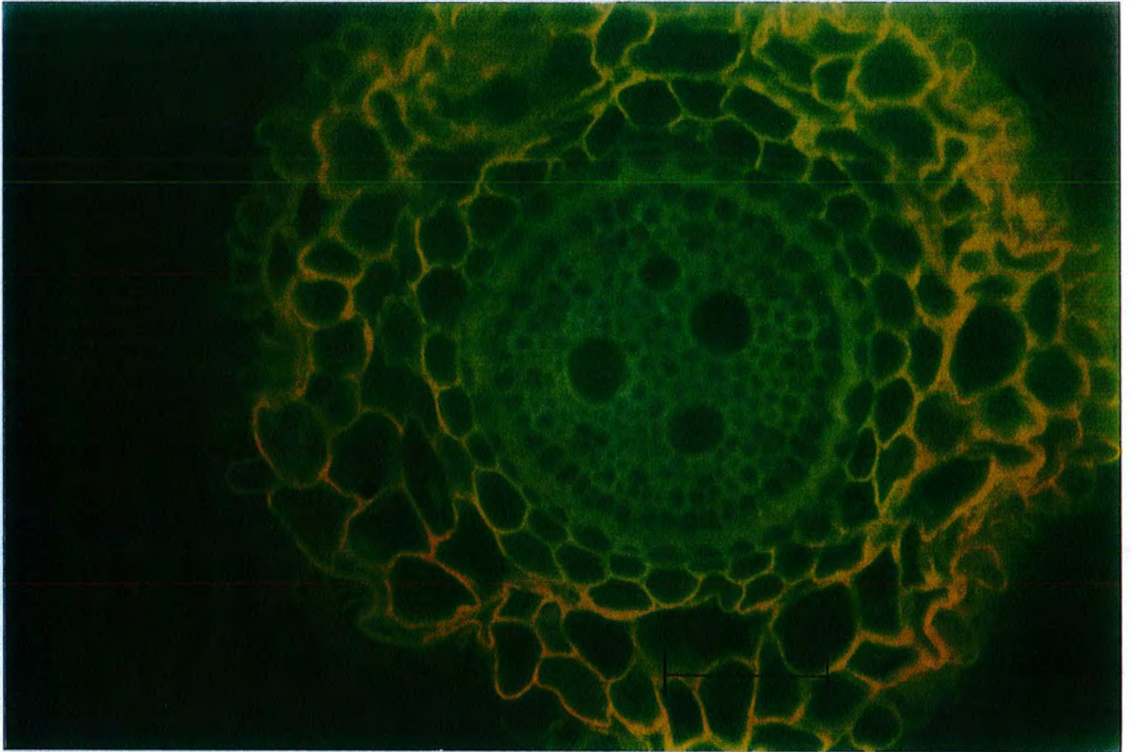
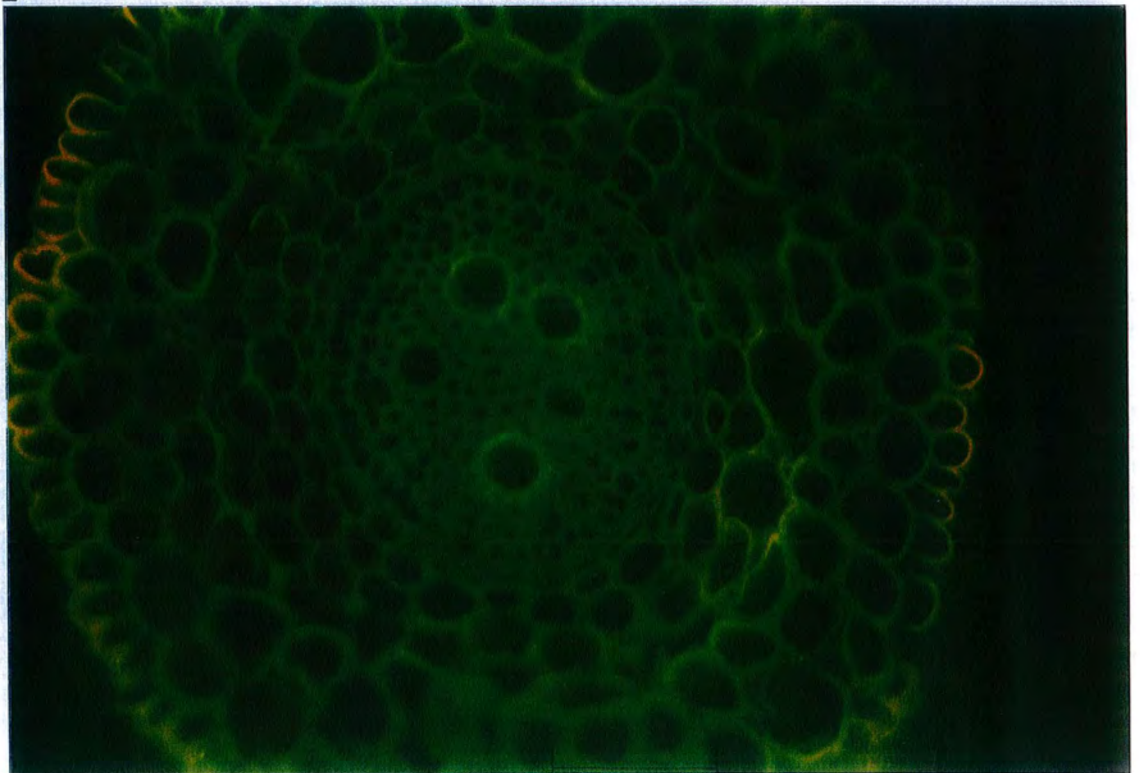


Plate 42



Plates 43 and 44 illustrate in detail the typical fluorescent deposition in the cell walls of the Cycocel pre-treated root cortex, when grown in both treatments (NS and NS +PEG). Here it is clearly seen that the fluorescent AO dye was deposited in both treatments, but it was much more pronounced in the NS + PEG conditions. Therefore, the combination of Cycocel and PEG treatments did enhance deposition into the walls which could be related to water retention in these plants.

In addition to the apparent changes in the cortex, It was noted that when the root samples of pre-treated GEN1 plants were stained with AO and grown in NS solution alone, a brighter fluorescent deposition in the endodermis was seen (Plate 45 and 46). The vascular tissue of the treated plants was also more heavily stained than that of the control plants. The significance of this was not clear in terms of potential water retention, however, depositions in the walls could impede water movement out of the roots to the osmotic hydroponic solution.

Plate 43

Section of root of the Cycocel pre-treated GEN1 plant, illustrating the root cortex, sampled from the hydroponic NS solution alone. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X100 with a blue-violet filter. Scale bar = 100 *um*.

Plate 44

Section of root of the Cycocel pre-treated GEN1 plant, illustrating the root cortex, sampled from the hydroponic NS + PEG solution. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X100 with a blue-violet filter. Scale bar = 100 *um*.

Plate 43

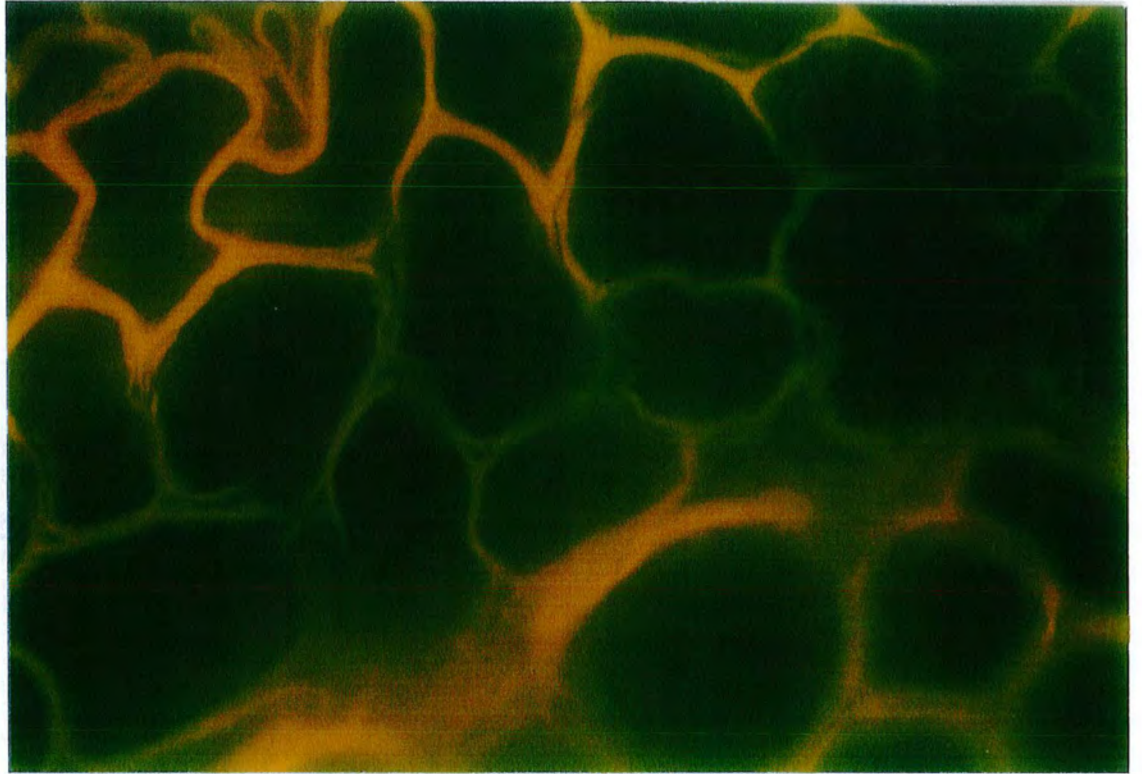


Plate 44

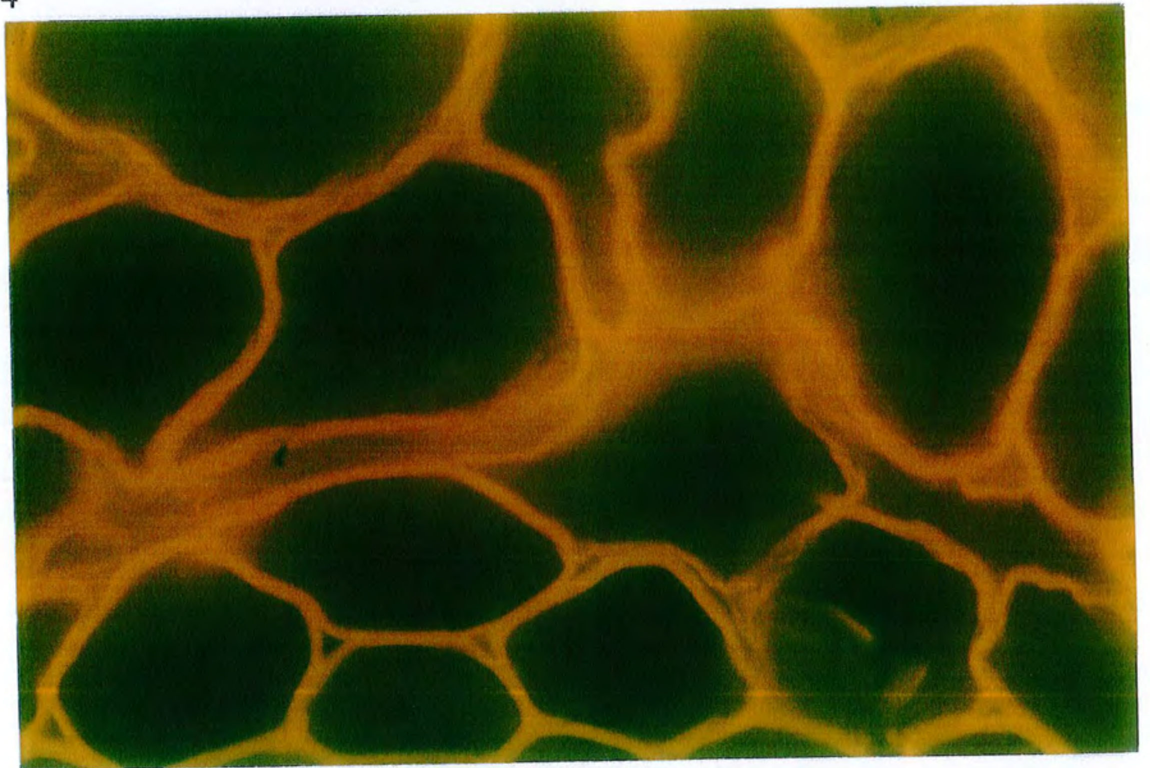


Plate 45

Section of root of the Cycocel pre-treated GEN1 plant, illustrating the endodermis, sampled from the hydroponic NS solution alone. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter.

Plate 46

Section of root of the control (untreated) GEN1 plant, illustrating the endodermis, sampled from the hydroponic NS solution alone. Specimens were stained with acridine orange, viewed and photographed under the illumination of the fluorescent microscope at a magnification of X20 with a blue-violet filter. Scale bar = 100 *um*.

Plate 45

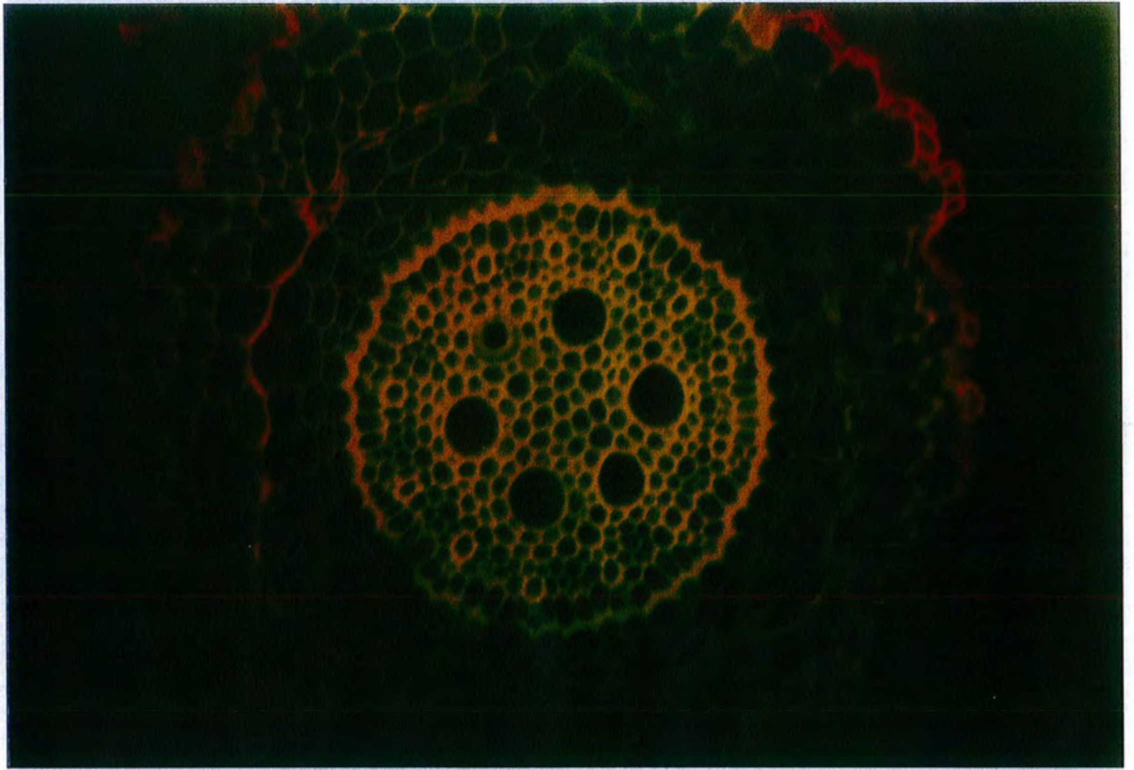
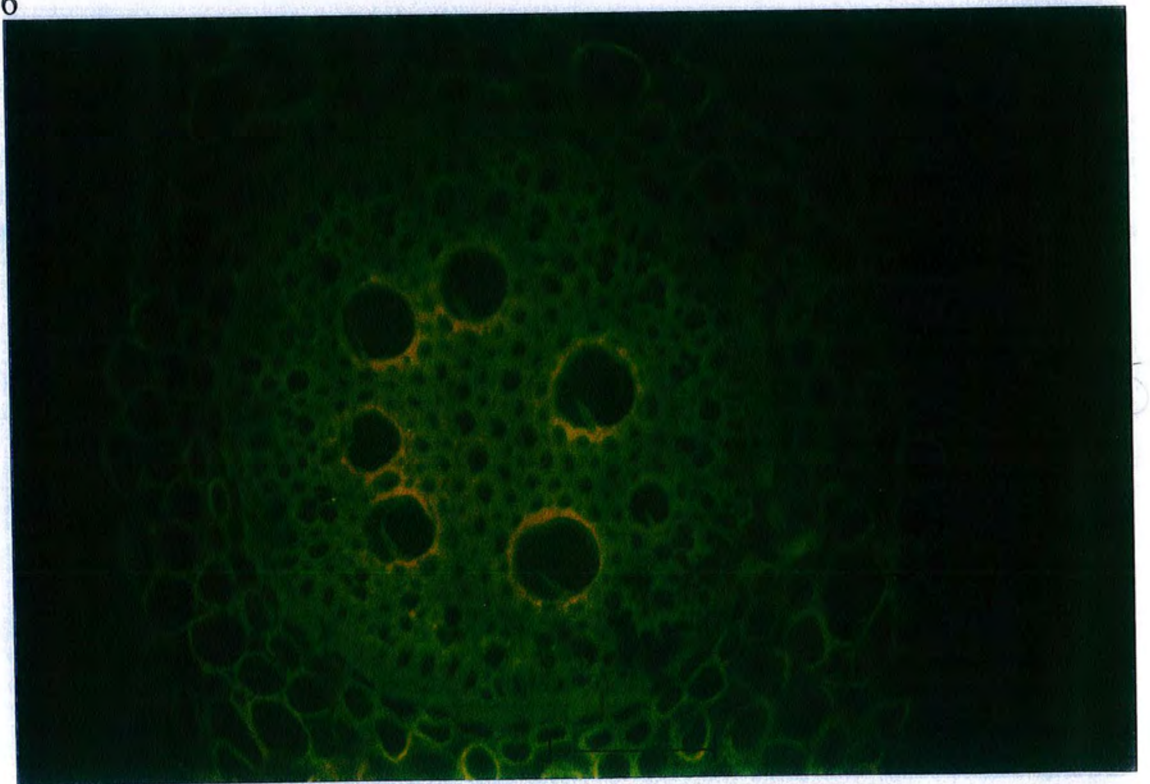


Plate 46



GENERAL DISCUSSION

Growth and development of a plant is mainly associated with environmental factors, which affect the physiological appearance of the plant. It has been noted earlier that environmental factors, such as high temperature and water limitation greatly affect a plant's development. In many cases, the growth, as observed by the elongation of stems and cell differentiations, can be manipulated either by the application of plant growth regulators (PGRs) or by breeding. For example, it has been reported by Pinthus, (1973) that plant breeding has successfully developed lodging tolerant cultivars, but has not abolished totally the problem of lodging.

PGRs are considered as an essential component in agriculture production and crop manipulation (Gianfagna, 1995). Cycocel, a synthetic plant growth regulator, has been shown by many investigators to retard growth, prevent lodging, enhance drought tolerance, promote flowering development and increase yield (Tolbert, 1960; Halevy and Kessler, 1963; Humphries, 1963; Hampton, 1986; Amoabin *et al.*, 1986; Abdulah *et al.*, 1978; Harris, 1978; Mathews and Culdicott, 1981; Bishnoi *et al.*, 1994; Olumekun, 1996). However, most of the investigative work has been confined to the use of the compound in a foliage application manner. Most of these investigation agree that Cycocel treatment to plants produces shorter, thicker, and greener leaves.

The rising concerns to minimise chemical applications to crops, and to the unfavourable occurring environmental changes, especially high temperature and limiting water availability, prompted a study of Cycocel application on wheat caryopses and any subsequent enhancement of the performance of the plants. It was considered that this early stage in the life cycle of the plant was an appropriate point for chemical

manipulation since enhancement of the critical stage of seedling establishment could be obtained.

Treatment of wheat caryopses by soaking them in a solution of Cycocel at concentrations of 0.004 and 0.04 % overnight, allowed a normal caryopses germination rate relative to the control. However, when the caryopses were soaked in 0.4 % Cycocel solution for the same period of time, the germination rate was dramatically delayed. There was no indication of toxicity symptoms caused to the caryopses nor the plants, i.e. allowing caryopses to germinate for longer time seedlings emerged almost to the same extent as the controls. The ability of Cycocel to inhibit seed germination has also been reported in the work of Wittwer and Tolbert, (1960) with lettuce seeds. In addition, earlier reports by Tolbert, (1960) stated that there was no toxicity symptoms seen with foliar application of 10^{-2} M (0.16 %) Cycocel to the wheat plants. Similarly Pinthus, (1968) stated that there was no harmful effect seen in association with the Cycocel treatment when applied at different growth stages to a number of wheat varieties or on their progenies. Cycocel was also reported by Hampton, (1988) to have no residual effect on the grassland Maku.

From these preliminary trial experiments it was seen that at the concentration of 0.4 % Cycocel pre-treatment of caryopses resulted in a prolonged tolerance of the wheat seedlings to water limitation. The plants, at the mature 4th leaf stage in soil, remained turgid and green for about 23 days after water was withheld; the water content of the plants was retained well above the level found in the controls or in the soil (Section I). This is in agreement with similar work of Halevy and Kessler, (1963) with bean plants where they found that the Cycocel treatment of the soil gave bean plants grown in it the ability to grow for 22 days longer than the controls after cessation of water. It was also noted in this current study with wheat, that when the

plants were re-watered, after 23 days of water withholding, they recovered, grew on and reproduced. These plants remained vegetative into the second year. Prolonged persistence of plants after Cycocel treatments was also reported by Ojeda and Trione, (1994) for guayule plants (*Parthenium argentatum* Gray) and Tabora and Hampton (1992) for Grasslands Maku. In this respect several experiments were initiated in order to investigate the Cycocel pre-treatment on the wheat seedlings of GEN1 plants and its potential carryover effect onto GEN2 plants.

The action of Cycocel may be attributed to the ability of the compound to penetrate the wheat caryopses and counteract the biosynthesis of GAs. Whilst the GAs are reported to activate the α-amylase enzyme in the aleurone layer of the caryopses (Chrispeels and Varner, 1967), counteracting this function will delay the production of simpler sugars and other solutes needed for the growing embryo. In light of the earlier results where seedlings emerged from caryopses treated with 0.4 % Cycocel this concentration was used routinely, in the rest of this study to examine its effect on the wheat seedlings.

Pre-soaking of the wheat caryopses with an aqueous solution of 0.4 % Cycocel influenced the seedlings' growth and developments. The major growth difference was the development of shorter plants (stem shortening) with thicker stem (wider diameter), and with leaves which were reduced in size with a darker green colour. The plant shortening may be related to the observed stunting of the first three internodes, in agreement with Olumekun, (1996), who stated that a foliar application of Cycocel, at the beginning of stem elongation, reduced the culm which was associated with the reduction of the first three internodes. This may be related to the retardation of cell division and elongation (Pinthus, 1968) and again can be related to changes in GAs biosynthesis. It also gave plants the ability to grow erect and prevent lodging.

However, stem elongation was observed to occur normally at later stages. These results suggested that pre-treatment of the caryopses resulted in a similar effect to that of the foliar applications as been reported in many investigators' work (Tolbert, 1960; Cathey and Stuart, 1961; Olumekun, 1996).

The results obtained from the Cycocel treatment on the growth of wheat seedlings under the 7-day watering regime showed that pre-soaking of the caryopses induced a hardiness to the environmental stress in terms of resistance to water limitation. Leaves of the untreated wheat caryopses showed a decrease in overall growth when the seedlings were subjected to water withholding regime of 7-day watering. In the long run, the growth of these plants became not suitable for any analyses. However, the combination of Cycocel and water withholding treatment was observed to enhance the growth, when subjected to the same watering regimes. It was also noted that there was an interaction effect between the Cycocel and the water limitation [(Figure 4) Section I]. An explanation for this observation is not immediately apparent. However, it appears that Cycocel pre-treatment preconditions plants to respond by enhancing or maintaining growth when the plant is subjected to water stress conditions.

The effect of Cycocel was apparent in reducing the size rather than changing the rate of appearance of leaves. It was also noted that Cycocel reduced the overall rate of growth of plant. These results are similar to the findings of many investigators' work on a variety of crops (Bruinsma, 1982; Weidner, 1987; Olumekum, 1996). Therefore at the morphological level, pre-treated seedlings were able to grow and enhanced the capacity to withstand the effects of induced water stress. It was also observed that the growth effect diminished as the plants aged, i.e. the influence of Cycocel was reduced probably by the breakdown of the chemical within the plants (not

rapidly destroyed). A similar observation was made by Tolbert, (1960) for foliar application of Cycocel in wheat. In the treated plants, tillers were also observed to be initiate very rapidly, and in greater quantity compared to the control plants. However, the results of Cycocel ability to enhance tillers initiation is not presented in this work only in as much that some carryover of resistance to water limitation was seen in them (Table 2). It is also widely reported that foliar application of Cycocel increases the number of tillers, but it is not known, if it increases the yield or the survival of tillers (Matthews *et al.*, 1981; Kettlewell *et al.*, 1983; Waddington and Cartwright, 1986; Ma and Smith, 1992).

One of the most striking effects of Cycocel was the reduced leaf size. This reduction of the leaf size was compensated for by an increased chlorophyll level. This increase may be related to a maintained photosynthetic capacity and the prolonged period of leaf senescence. A similar observation was also reported by Ojeda and Trione, (1994) from the treatment of Cycocel to the *Parthenium argentatum* Gray plants. In this current study on wheat with low Cycocel concentrations, 0.004 and 0.04 %, it appeared that chlorophyll per g dry weight was decreased when subjected to 4-day watering compared with the 2-day watering regime. Caryopses pre-treated with 0.04 % Cycocel showed a significant increase in chlorophyll level up to the 3rd leaf, but not with the 4th . However, at this concentration, Cycocel appeared not to counter the low level of chlorophyll when subjected to the moderate water limitation. The individual level of chlorophyll a and chlorophyll b was also the same as for the total chlorophyll. There was no differential effect seen for watering regime itself and for the combination of Cycocel. Therefore, some inconsistency of response of different parts of the plant was observed - the effect of Cycocel is complex and may influence a

number of physiological and biochemical processes in plants, either directly or indirectly.

The widespread effect of water stress on the accumulation of proline in many plant species has been well documented. Proline is probably the most widely distributed compatible solute in relation to plant stress. Several studies have considered its role as an osmotic regulator in relation to water stress (Levitt, 1980; Miflin, 1980). For example, it has been reported that proline is capable to accumulates quickly and extensively during a water limitation (Singh *et al.*, 1973), and its accumulation is dependent upon plant species, organ, growth stage ant water availability (Srivastava and Singh, 1987). It has also been noted that proline often accumulates in leaves grown under water stress (Al-Karaki *et al.*, 1996) and under various stress conditions (Milfin, 1980). Free proline has been indicated by Aspinal and Paleg, (1981) to accumulate in plant tissues in response to drought stress, which results in a level of a 10 - 100 fold increase in the amount of proline in leaves. There are some indications that proline may serve as an initial protective effect for stress tolerance in the majority of crop plants that have been studied (Stewart and Larher,1980), which may be injury at higher level (Levitt 1980; Salisbury and Ross 1985). However, most of the studies of proline accumulation in plants were concerned with plant stress physiology (Amberger-Ochsenbauer and Obendorfer, 1988) and considerably less known of its accumulation in response to growth regulator applications. The potential for Cycocel to act as a protectent against water limitation through proline level manipulation was investigated.

Analysis of proline accumulation in the pre-treated wheat seedlings suggested that Cycocel, under a continuous watering regime, had no direct influence on the proline accumulation. However, when the same plants where subjected to 7-day water

limitation proline was detected. Likewise, prolonging the drought treatment for about 20 days prior to the extraction resulted in proline detection in the leaves of the pre-treated plants. In view of the proline results, Cycocel pre-treatment does not influence proline accumulation directly, this occurs only during water stress, as the case in the untreated plants which subjected to water limitations. The results suggested that Cycocel pre-treatment may have preconditioned the plants for the production of amino acids whilst not having a direct effect on proline accumulation. However, since no quantitative estimation of proline was made in this study it was not known if Cycocel enhanced the amount of proline in the leaves under water stress.

Modification of water loss in leaves can be achieved through alteration of stomatal functions or patterns. It has been reported by Meidner and Mansfield, (1968) that guard cells movements are extremely influenced by the water supply to the plants. Therefore any unfavourable changes in water availability to plants would depress day time stomatal conductance (Davis *et al.*, 1981), and with a more severe water limitation the stomata may close throughout the day (Weyers and Meidner, 1990). On the other hand, it should be noted that several investigators' work have stated that stomata of drought-hardened leaves of maize, sorghum, cotton and wheat remained open at low water potential, resulting in a relatively high photosynthetic rate which could be related to lowering of stomatal sensitivity under stress conditions.

Moreover it has also been noticed that water limitation not only affects the morphological characteristics of cotton strains (Burke *et al.*, 1985), but it also may have an effect on the size or the response of individual stomatal characteristics of sorghum crop plant (McCree and Davis, 1974). In addition, water stress has also been

reported to influence the cell division, cell expansion and the primordium development of many plants (Slatyer, 1967; Hsaio, 1973).

Experimental work with plant growth regulators has been shown to influence the stomatal function, i.e. abscisic acid (ABA) has been reported to regulate the closure and opening of stomata during water stress (Quarrie and Jones, 1977; Grantz, *et* al., 1985). It has also been indicated by Wright, (1969) that ABA concentration increases with an increase in the level of water deficit. This alteration is associated with stomatal closure, which is considered the main cause of the transpiration decline (Hsiao, 1973). In addition to their effects on stomatal function it is also possible that growth regulators may influence the development and distribution of stomata in leaves.

The manipulation of stomatal patterns by Cycocel to distinguish between the effects on stomatal patterns and effects on growth is not altogether clear (Amoabin *et al.*, 1986). Thus the potential to enhance water limitation tolerance by Cycocel treatment through the manipulation of stomatal patterns was initiated.

The results from the pre-treatment of caryopses with the growth substance on stomata was found to be inconsistent in its effect on the stomatal pore, Stomatal Density and the epidermal cells. Treatment did not appear to give a significant change consistent with an ability to limit water loss from plants. The size of the epidermal cell was also not changed, i.e. epidermal cells were present in almost equal numbers of cells per mm^2 from Cycocel treated and untreated plants. From the present investigation it was clear that neither Cycocel as Cyanamid nor the commercial Cycocel showed a clear pattern of response which would be counted with a retention of water in plants. These findings contradict the work of Gupta, (1992) with the *Vicia faba* L. which indicated that at higher concentrations of Cycocel treatment to the seed increased the epidermal cells and stomatal size, while it decreased the Stomatal Index. This

discrepancy in the results of the effect of the growth substance adds confirmation to the statement of Tabora and Hampton, (1992) that Cycocel may be species-specific and may be even cultivar specific. This is also in agreement with Singh *et al.*, (1973) who suggested that barley crops are less responsive to Cycocel, and require a higher concentration of the Cycocel treatment than other species. Moreover, the variability in response of the plants to the applied Cycocel may be related to one, or all, of the following, (a) selectivity of the plant organs, (b) concentration and method in use, (c) duration of treatment, and (d) environmental conditions (Tolbert, 1960; Halevy *et al.*, 1966; Bode and Wild, 1984; Harker and Taylor, 1994).

In many cases, changes in plant morphology, due to environmental factors, is reflected in a relative change in the root system so that an equilibrium in the rate of growth is maintained compared to the shoot (Richards, 1980; Troughton, 1980). It is also recognised that to achieve efficient plant growth a high value of root growth may be necessary (Harper, 1983). Pugnair *et al.*, (1994) stated that an increase in root/shoot ratio is an adaptive process of the plant in response to drought stress. Limitation was involved within pot experiments in the manipulation of water stress conditions in relation to root growth. Therefore the influence of Cycocel pre-treatment on wheat seedlings was investigated through the use of honey jar hydroponic treatments in conjunction with PEG.

In this current work, similarity in plant growth responses were seen in the culture solution treatments, as in the case of the pot experiments. Here again there was a concomitant shortening of the stem, and production of short dark green leaves in response to Cycocel pre-treatments.

Induced water stress (through PEG treatment) inhibited both apex and leaf elongation. The growth retardant, however, modified the plant growth responses. It

appeared that Cycocel pre-treatment counteracted the effect of the PEG-induced water stress, i.e. the growth retardant have enhanced the growth of 3rd and 4th leaves in the NS + PEG solution, when compared to their untreated counterpart (Figure 32, Section II). This response was similar to that of the pot experiment for plants subjected to a 7-day watering treatment (Figure 5, Section I). According to Singh *et al.*, (1973) and Bishnio, (1994) Cycocel modified the developmental growth response of the plant to water stress. This alteration effect of Cycocel could be related to the modification of water uptake by plants, changes in the rate of water loss through transpiration, and/or affecting the balance force of the water entering and within plant cells.

In contrast to the changes caused by the induced water stress on the vegetative growing plant parts, Cycocel treatment has also been reported to have a striking effect on the plant growth development. However, this reduction in stem extension has been shown by many investigator as not to be an effect on the total dry matter accumulation of wheat plants (Tolbert, 1960; Bragg *et al.*, 1984; Green *et al.*, 1986). Olumekun, (1996) stated that reduction in stem elongation was not proportionally reflected as a reduction in total dry matter accumulation. Thus the influence of Cycocel coupled with PEG on the fresh and dry weight of wheat seedlings were initiated. In this study, under the NS treatment alone, Cycocel reduced both fresh and dry weight of shoots 35 days after planting as compared with the control seedlings. However, under the PEG treatments of Cycocel pre-treated plants, fresh and dry weight values were maintained. This again indicate that Cycocel was able to counteract water stress in maintaining growth. On the other hand, under PEG treatment the fresh and dry weight of the shoots of the control plants were reduced.

The measurement of fresh and dry weight of root systems, from the same treatments, were also made. Under the PEG treatments fresh weight of the control

plants' roots was reduced, which is consistent with a reduction in cell size. This may suggest that cells of the root tissues were impaired in their growth so that water uptake was inhibited. However, dry weight of these roots was increased, which reflects an increase in overall root tissues in terms of cells. This would be expected as a response to water stress where increased potential root area would be noted. In contrast, Cycocel pre-treatment did maintain the fresh weight of the roots under the PEG-induced water stress, with no difference seen in the dry weight of these roots and the values was enhanced over the NS grown roots. Here again Cycocel either enhanced the water uptake component of the root tissues, or maintained the tissues water availability, i.e. restricting water loss through the tissues membrane so that a water equilibrium could not be reached with the osmotic medium.

It has been well documented that prolonged water stress affects virtually all metabolic processes. This may result in a severe reduction in plant productivity (Bohnert *et al.*, 1995). Despite the considerable effects of water shortage in the plants' metabolic process, free proline, a compatible solute, is known to accumulate in plants as an initial protection against drought. The effect of Cycocel pre-treatment on proline accumulation in wheat seedlings along with PEG-induced water stress was investigated. In this study, the analyses of amino acids extraction included both shoots and roots, since hydroponic culture allowed this.

As in the case of the pot experiments, when the amino acids were analysed by TLC, Cycocel pre-treatment did not show proline accumulations in the wheat 4th leaf in NS solution. Unlike the pot experiments, however, subjecting either the pre-treated or the control seedlings to PEG-induced water stress at -5 bar did not result in the accumulation of proline. Therefore at -5 bar Cycocel treatment had no effect on proline accumulation either in the presence or absence of induced water stress. In

contrast Singh *et al.*, (1973) detected proline in wheat plants when they were subjected to water stress imposed by flooding the seedling pots with PEG solution at -10 and -20 bars. They indicated that proline accumulation rate was very slow in the moderate water stress, but Cycocel did not affect the free proline accumulation in wheat seedlings. Proline accumulation in the Cycocel treated-plants, under PEG-induced water stress, was also reported by Agarwal *et al.*, (1994) and Handa *et al.*, (1983). In this study it may be that the treatment was not severe enough, however, this concentration of PEG did have an effect in the growth of plants. Alternatively the plants may have adjusted to water stress by the time proline analysis was made so that accumulation of this compound was not detected.

In addition, in this study, pre-treated seedlings subjected to PEG treatment appeared to exhibit a production of an extra purple staining band detected by TLC, which did not appear to have the proline characteristics (Figure 41, Section II). The identity and significance of this compound was not resolved.

The analysis of the root system under NS solution alone and NS + PEG treatments did not appear to indicate any proline accumulation for either the control or the pre-treated plants. Interestingly, analyses of the amino acids in both treatments under the PEG-induced water stress produced similar patterns of band constantly under TLC analysis. However, the absolute R*f* values were variable for many of the bands of the shoots and roots. This may have been due to high salt availability to the plants under these growth conditions which interfered with the analysis. Therefore in this study, at -5 bar osmotic potential, proline was not produced under any treatment in hydroponic culture.

Further investigation of the Cycocel pre-treatment on stomatal characteristics (stomatal pore length, Stomatal Density and Index, and epidermal cells density) was made in relationship to NS and NS + PEG hydroponic treatments.

Unlike the results obtained from the pot experiments, under NS solution, Cycocel pre-treatment did reduce the length of stomatal pores present in the 4th leaf. This may be due to the Cycocel ability to stunt overall growth, however, this relationship (reduced stomatal pore size) was not maintained when the plants were subjected to PEG-induced stress. There was no significant difference ($P< 0.05$) found in the stomatal pore length between the control and Cycocel pre-treated plants grown in PEG.

It was also found that Cycocel pre-treatment did increase the Stomatal Density over the control when grown in both NS and NS + PEG at -5 bar, but this was not linked to a reciprocal decrease in pore size. On the other hand, Cycocel pre-treatment appeared not to alter the epidermal cell number (per given area). Therefore, as was the case for the pot experiments, it appears that Stomatal Density alteration was a results of an increased changes in stomatal size rather than the epidermal cells.

Pre-treatment of the seedlings in NS and NS + PEG increased the Stomatal Indices over the control, but PEG-induced water stress did not enhance this parameter. This would be the opposite to what would be expected for a reduction in water loss. The changes in Stomatal Index would indicate an effect on cell differentiation rather than growth.

The long-term effects of Cycocel pre-treatments on subsequent generations (carryover effects of the treatments) was also investigated. The caryopses of GEN2

plants derived from the GEN1 Cycocel pre-treated generation were not exposed to any Cycocel treatment themselves. Exposure of GEN2 seedlings to 15 days water withholding showed that they have an ability to retain water slightly higher than the control GEN1 plants. This was not to the same capacity as to the water retained in GEN1 plants, which grew from the pre-treated caryopses. It appeared that Cycocel pre-treatment was even able to enhance water retention in the GEN2 generation. Furthermore, under the 7-day watering regime seedlings appeared to accumulate more water than the 2-day watering. Here again, as in GEN1 seedlings, the ability to respond to water stress was initiated by that treatment itself.

Leaves of the GEN2 seedlings, derived from GEN1 seedlings, were not affected by the watering regimes in terms of overall growth. It was found that neither the 2-nor the 7-day watering regime altered the leaves of the seedlings, unlike the response of the GEN1 seedlings. It appeared that Cycocel pre-treatment of GEN1 seedlings had modified the characteristics of the second generation GEN2 cell tissues.

As noted earlier, Cycocel pre-treatment was capable of preserving the chlorophyll level in the GEN1 leaves. The GEN2 leaves were also able to maintain the chlorophyll level slightly more than the untreated GEN1 leaves, when subjected to the 2-day watering regime. In addition, when the untreated GEN1 seedlings were subjected to 7-day watering regime, the chlorophyll level was significantly ($P = 0.05$) decreased, whereas the GEN2 seedlings preserved their chlorophyll under the same treatment conditions.

Overall, it was noted that GEN2 plants contained slightly more total chlorophyll, and individual chlorophyll a and chlorophyll b when extracted from the 3rd and 4th leaves than the GEN1 controls. This could be related to the ability of GEN2 seedlings to survive water limitation as measured by the chlorophyll content.

However, when chlorophyll was extracted from the GEN2 4th leaf tissues, a higher proportion of the total chlorophyll was left behind as shown in Section III. This also suggests that anatomical changes may have been taken place in tissues in response to Cycocel pre-treatment of the GEN1 caryopses. It prevented chlorophyll release, maintained the leaf size of the GEN2 seedlings under the moderate water limitation (Figure 50, Section III), and also prevented wilting. In the light of these observations an anatomical examination of the wheat leaves was initiated to localise the chlorophyll present.

In this current investigation it was found, after extraction, there was some residual chlorophyll left behind in the untreated GEN1 seedlings, which was not visible. The cells of the leaves of the same seedlings were collapsed and the tissues distorted. In contrast, the leaf structure of the GEN2 seedlings appeared to be fully turgid, cells did not collapse, and the leaf tissues were not distorted, under both watering regimes. Additionally, large amount of chlorophyll appeared to be present as indicated by the bright red fluorescence of the cells. This again confirms that the Cycocel pre-treatments may have modified the cell structure of the seedlings by the enhancement of the cell wall structure, i.e. more rigid cell wall structure which preserve the release of the chlorophyll by the acetone extraction.

Since GEN2 seedlings were able to withstand water limitation better than the untreated GEN1 seedlings, it was regarded as appropriate to investigate the Cycocel pre-treatment influence on the GEN2 stomatal characteristics. The analysis of the 3rd and 4th leaves of the GEN2 seedlings showed variability in stomatal characteristics within the same plant. However, there was a constant pattern of stomatal pore length between the 3rd and the 4th leaf grown under 2 and 7-day watering regime, in that watering regime had no effect on the stomatal length, i.e. under the 7-day watering

regime, the stomatal length of either the 3rd or the 4th was not altered. This was contrary to what was expected as an appropriate protective mechanism of stomatal characteristics against water limitations where stomata could have been smaller. It also opposed the statement of McCree and Davis, (1974) that water limitation may have an effect on the stomatal size of sorghum crops.

The stomatal similarity in pattern length and its persistence under the 7-day watering treatments may be induced by the changes caused, in GEN1 seedlings, by the Cycocel pre-treatments of caryopses. This may be regarded as an essential behaviour of the stomata, i.e. there was almost an equal sensitivity effect of stomata under both watering treatments (2 and 7-day watering regimes) where any pre-conditioning of the plants had already taken place in the GEN1 generation.

The Stomatal Density and Index of the 3rd and 4th Leaves of the GEN2 seedlings did not show any statistical differences when grown under the 2 and 7-day watering treatments. There was some variation between the two leaves but this was not statistically significant ($P = 0.05$). Epidermal cell density was also found to be not significantly different for both leaves for both watering regimes. It appeared that the response of Stomatal Density, Index and epidermal cells of the GEN2 seedlings to watering treatment is minimal, if any. Therefore, from the results obtained, it would appear unlikely that stomatal characteristic would contribute to any limitation of water loss.

Since the root and shoot ratio is an important indication of the water stress effects, here again the need of a finer control of water limitation treatment and access to roots in an intact state was found inevitable. Therefore an investigation of the

relationship between GEN2 seedlings and a possible Cycocel carryover effect on the fresh and dry weight was initiated using the hydroponic culture (NS and NS + PEG at -5 bar). The GEN2 seedlings were harvested after about 30 days of growth.

Growing the GEN2 seedlings in NS alone resulted in an increase of the shoots fresh weight when compared to its comparable dry weight. This was higher than its corresponding GEN1 seedlings under the same treatments. However, the values of both GEN2 and GEN1 seedlings fresh and dry weight under PEG-induced water stress were reduced, but were not statistically significant ($P = 0.05$). The analysis of fresh and dry weight of roots indicated an almost identical pattern to the values obtained for the shoots. However, under NS + PEG treatment, the fresh and dry weight of GEN2 roots was slightly increased over the control under the same treatment, but was not statistically significant ($P = 0.05$). Here there appeared to be some response of the GEN2 seedlings to the Cycocel as a carryover effect. It is similar to those in response to Cycocel treatment of GEN1 caryopses.

Because of the nature of the results achieved from the GEN1 (Cycocel pre-treated) and the GEN2 seedlings in relationship to drought stress, it appeared that changes in the anatomy of the tissues could be important in the Cycocel response. The leaf anatomy and the comparative root anatomy of the Cycocel pre-treated GEN1 and GEN2 (derived from GEN1) seedlings was described from transverse sections of the leaf and the thin slice section of roots, with the objective of identifying the effect of Cycocel on the cuticle thickness changes, cell wall deposition and cell size related to stomatal patterns. As stated earlier, this work established a further contribution to the physiological analyses, showing an overview of the anatomical appearance of the Cycocel pre-treatment influence on wheat leaves and roots. Because of lack of similar

anatomical information of the influence of Cycocel, it has been difficult to relate this work to other investigators works for the comparative reasoning.

Tissue of the cells of the control GEN1 leaves, sampled from the middle portion of the leaf and stained with calcofluor, revealed no obvious differences in the wall thickness of the epidermal cell in particular, and there was no obvious cuticle in the tissue for comparison between the two watering treatments. On the other hand, fluorescence of the brightner under the UV barrier was observed for the cell wall of the GEN2 seedlings. The cell walls were also not collapsed and wall structure appeared to be more rigid in comparison to the GEN1 control seedlings indicating resistance to water limitation in cell structure. Here it appeared that cell walls retained rigidity which could have accounted for the lack of wilting.

Anatomical examination of changes, if any, within the full leaf blade were also investigated from the tip, mid, and base region of the GEN2 plant leaves, under both 2 and 7-day watering regimes. Similar red fluorescence was observed for all 3 segments of the GEN1 and GEN2 plant leaves, under the 2 and 7-day watering treatments when viewed under a UV illumination. This indicates that chlorophyll levels were unchanged under the two treatments. However, a notable difference between the GEN1 and GEN2 plants was seen in the presence of an enhanced blue fluorescing deposition in the upper and lower epidermal cells of the mid and base section of the GEN2 leaves (Plates 20 and 22). These was also seen in the GEN1 7-day watered plants, but not with the 2-day. There changes could be related to watering regimes, but were more pronounced in the GEN2 leaves. The identity of this deposition is not known but it may have consisted of cellulose material which could alter wall permeability properties.

A similar analysis of the root anatomy of plants growth with and without Cycocel pre-treatment under different watering regimes was not totally conclusive.

However, some differences in staining patterns were observed which could be related to an anatomical modification in response to water limitation. The cortex of roots from pre-treated GEN1 plants under NS treatment alone indicated a dense bright fluorescing deposition, however, examination of roots of untreated GEN1 plants showed a slight scattered deposition in the cortex. On the other hand, when root tissues from NS + PEG treatments were examined, the deposition in the Cycocel pre-treated root cortex was much more apparent over the untreated GEN1 cortex. However, the latter did have slight fluorescence in the control. From the NS alone, it appeared that Cycocel pre-treatment may have modified the roots. However, this modification was strongly enhanced when the plants were grown under the PEG-induced water stress. This deposition was not identified but it is possible that its inclusion in the walls of the cortical cells could have enhanced water-holding, or uptake, capacity, which could explain the water retention in these plants. Similarly, the slight yellow fluorescing deposition in the untreated plants under the same PEG treatment may also explain a modification to the roots before it goes into total collapse.

In addition, epidermal cells of the pre-treated GEN1 plant roots, when grown under both treatments (NS and NS + PEG), indicated a fluorescing deposition of the acridine orange (AO). This deposition was highly pronounced in the PEG-induced water stress. This confirms that Cycocel and PEG combination did enhance deposition in the walls. Additionally, deposition of the acridine orange complex was also noted in the endodermis, and vascular tissue of the treated plants, however, the significant of this in relation to water retention is not clearly known.

From these observation it can be seen that Cycocel pre-treatment modified cell wall structure in the cortex of roots. Again also it appears that Cycocel pre-treatment preconditions the plants to a water stress resistance response.

It is apparent from this study that pre-treatment of caryopses with Cycocel enhanced the resistance of seedlings derived from them to water limitation treatments. It should be noted, however, that the seeds used for the GEN 1 control for the comparison of GEN1 and GEN2 response to drought had, unlike the GEN 2 seeds, not been subjected to drought themselves during their growth and development. Consequently, some unknown effect of drought during seed development in the GEN1 might have influenced plant response to drought in the GEN 2 plants that were grown from these seeds.

This enhanced resistance to drought is particularly important in seedling establishment which is a critical and sensitive stage in the life of a plant. The studies here were all conducted using plants grown under controlled environmental conditions and the responses and results are specific to those conditions. For the agricultural potential of the effects of Cycocel to be appreciated fully then field experiments would need to be conducted.

Some morphological, anatomical, and physiological observations were made on changes in treated plants which could account for this ability. Clearly this study has not been conclusive, further investigations could be initiated, for example, in order to identify the nature and function of the deposition of the roots. also the preconditioning response could be investigated at a genetic level. A genetical study would be particularly relevant in investigating the response, and apparent carryover effect in the GEN2 generation.

Conclusions

1) Imbibing the caryopses of wheat with 0.4 % of (2-chloroethyl)-trimethylammonium chloride (Cycocel/CCC) delayed germination, but did not prevent it.
2) At the morphological level, the seedlings of wheat from pre-treated caryopses were stunted, mainly by the reduction of the first three internodes. This was consistent with the appearance of a wider stem, with shorter dark green leaves than controls.
3) The Cycocel treatment of caryopses allowed the seedling derived from them to survive water limitation treatments for up to 23 days, while the control was completely desiccated by then. Moreover, re-watering of the pre-treated seedlings allowed recovery of the plants which reproduced in the second year.
4) Cycocel was also able to enhance growth as a counter to water stress, by means of maintaining the fresh and dry weight accumulation of shoot tissues under PEG-induced water stress.
5) Pre-treatment also enhanced the water holding component of tissues.
6) Cycocel pre-treatment did not have a direct influence on proline accumulation, however, it appeared to have a pre-conditioning effect in that proline was accumulated when plants were subjected to water limitation..
7) Cycocel pre-treatments did not appear to modify the stomatal characteristics: stomatal pore length; stomatal density; epidermal cell

density, which would be compatible with a reduction in water loss from the leaves.

8) Cycocel enhancement of resistance to water limitation was apparent in the subsequent GEN2 seedlings derived from GEN1 plants from pre-treated caryopses. GEN2 plants survived for up to 15 day water withholding, showing some enhanced ability to retain water better than control (GEN1 seedlings).
9) A potential carryover effect of the Cycocel pre-treatments were apparent in the growth development of the GEN2 seedlings. It was apparent that GEN2 seedlings were less susceptible to drought than the control GEN1 seedlings.
10) The water stressed GEN2 plants retained chlorophyll within the 4th leaf tissues after 80 % acetone extraction. This was not seen in GEN1 plants, and may be attributable to the Cycocel pre-treatment effect on the tissues cell wall/membrane.
11) At the anatomical level, the leaf cell structure of the GEN2 plants appeared to be fully turgid, cells did not collapse, and tissue was not distorted, after 80 % acetone extraction, than control for both treatments (2 and 7-day).
12) The cortex of the GEN1 plants derived from pre-treated caryopses, grown in NS solution alone, indicated a bright fluorescing deposition. This deposition was enhanced in roots of plants grown under PEG-induced water stress conditions.

REFERENCES

Abdelhadi, W., J. L. Prioul and R. Brourdu (1980). Contrasting effect of (2-chloroethyl)trimethylammonium chloride (CCC) on photosynthetic gas exchange and carboxylase activites. Photosynthetica, 14: 437-440.

Abdulah, K. S., A. E. Canham and G. P. Harris (1978). Effects of CCC on the formation and abortion of flowers in the first inflorescence of tomato (*Lycopersicon esculentum* mill). Annals of Botany, 42: 617 - 625.

Adedipe, N. O., D P Ormond and A. R. Maurer (1968). Response of peas to soil and foliar application of Cycocel (2-chloroethyl)trimethylammonium chloride. Canad. J. Plant Sci., 48: 323-325.

Agarwal, R. M., S. Gupta, and K. Jeevaratnam (1994). NADH-dependent glutamate dehydrogenase activity in *Lablab purpureus* L. under polyethylene glycol '6000' induced stress, Cycocel and conditioning treatments. Indian J. of Experimental Biol., 32: 812-815.

Alaib, M. A. (1985). Seedling responses to growth regulators and drought stress. M.Sc. thesis, Biological Sci. Dep. Uni. of Durham.

Aldasoro, J. M., A. Mmatilla, and G. Nicolas (1981). Effect of ABA, fusicoccin and thiourea on germination and K^+ and glucose uptake in chick-pea seeds at different temperatures. Physiol. Plant., 53: 139-145.

Al-Karaki, G. N., R. B. Clark and C. Y. Sullivan (1996). Phosphorus nutrition and water stress effect on proline accumulation in sorghum and bean. J. Plant Physiol., 148: 745 - 751.

Allen, M. B. (1966). In: The chlorophylls. L. P. Vernon and G. R. Seely (eds.). pp: 511-519. Academic Press, New York.

Amberger-Ochsenbaure, S. and J. Obendorfer (1988). Level of free proline in ornamental plants: I. Influence of plant age, leaf age, and leaf region in saintpaulier and chrysanthemum. J. Plant Physiol., 132: 758 - 761.

Amoabin, S., L. G. Paleg and D. Aspinall (1986). The effect of (2-chloroethyl) trimethylammonium chloride (CCC) on stomatal diffusive resistance in tomato. J. Plant Physiol., 122: 437-444.

Aspinal, D., and L. G. Paleg (1981). Proline accumulation: physiological aspects. In: The Physiology and Biochemistry of Drought Resistance in Plants. L. G Paleg, and D. Aspinall (eds.). pp206 - 42. Academic Press.

Austin, R. B., J. Bingham, R. D. Blackwell, L. T. Evans, M. A. Ford, C. L. Morgan and M. Taylor (1980). Genetic improvement in winter wheat yields since 1900 and associated physiological changes. J. Agric. Sci. (Camb.), 94: 675-689.

Baldev, B., Lang, A. and A. Agatep (1965). Gibberellin production in pea seeds developing in excised pods: effect of growth retardants. Science. 147: 155-157.

Balki, A. S. and V. R. Padole (1982). Effect of pre-sowing seed treatments with plant hormones on wheat under conditions of soil salinity. J. Indian. Soil Sci., 30: 361-365.

Barnett, N. M. and A. W. Naylor (1966). Amino acid protein metabolism in Bermuda grass during water stress. Pl. Physiol., Lancaster 41: 1222-30.

Bates, L. S., R. P. Waldren and I. D. Teare (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Bhatt, J. G. and T. Ramanujam (1970). Effect of Cycocel on yield, chlorophyll content and fiber properties of lint of MCU cotton. Indian J. Plant Physiol. 13: 80-84.

Bishnoi, N. R., R. S. Karwasra, S. N. Gupta and C. R. Bainwal (1994). Influence of growth retardants on water relations and yield of Bajra under rainfed conditions. Indian J. Plant Physiol., 37(2): 73-75.

Bode, J. and A. Wild (1984). The influence of (2-chloroethyl)trimethylammonium chloride (CCC) on growth and photosynthetic metabolism of young wheat plants (*Triticum aestivum* L.). J. Plant Physiol., 116: 435-446.

Bohnert, H. J., D. E. Nelson and R. J. Jensen (1995). Adaptation to environmental stress. Plant Cell, 7: 1099-1111.

Bragg, P. L., F. K. G. Henderson, W. J. Fielding, and R. Q. Cannel (1984). A comparison of the root and shoot growth of winter barely and winter wheat, and the effect of an early application of chlormequat. J. Agric. Sci., Camb., 103: 257 - 264.

Bruinsma, J. S. (1982). Plant growth regulators in field crops. In: Chemical Manipulation of Crop Growth and Development. J. S. MacLaren (ed.). pp. 3-11. Butterworth Science, London.

Budavari, Susan, M. J. O'Neil, A. Smith and P. E. Heckelman (eds.) (1989). The Merck index: An Encyclopaedia of Chemical, Drugs, and Biologicals. 11th. (ed.). Merc. & Co. Inc. Publisher. N.J., USA.

Burger-Wiersma, T., M. Veenhuis, H. J. Korthals, C. C. M. Van de Wiel and L. R. Mur (1986). A new prokaryote containing chlorophylls a and b. Nature, 320: 262-4.

Burke, J. J., E. G. Patricia, L. L. Hatfield, and J. E. Quisenberry (1985). Plant morphological and biochemical responses to field water deficit. Plant Physiol., 79: 415 - 419.

Campbell, N. A. (1996). Photosynthesis. In: Biology 4th (ed.). The Benjamin/Cummings publishing, California, USA.

Cathey, H. M (1964). Physiology of growth retarding chemicals. Annual Review of Plant Physiology, 15: 271-302.

Cathey, H. M., and N. W. Stuart (1961). Comparative plant growth-retarding activity of AMO-1618, Phosfon, and CCC. Botanical Gazette. pp. 51-57.

Chrispeels, M. J. and J. E. Varner (1967). Hormonal control of enzyme synthesis: on the mode of action of gibberellic acid and abscisin in aleurone layers of Barley. Plant Physiol., 42: 1008 - 1016.

Cox, W. J. and D. J. Otis (1989). Growth and yield of winter wheat as influenced by chlormequat chloride and ethephon. Agronomy J., 81: 264-270.

Davis , W. J., J. A. Wilson, R. E. Sharp and O. Osonubi (1981). Control of stomatal behavior in water-stressed plants. In: Stomatal Physiology. Jarvis, P. G. and T. A. Mansfield (eds.). Cambridge University Press, pp: 163 - 185.

Delauney, A. J.D. and P. S. Verma (1993). Proline biosynthesis and osmoregulation in plants. The Plant Journal, 4: 215-23.

Dingle, J. T. and A. J. Barret (1969). Uptake of biological active substances by lysosomes. Proc. Roy. B. 173: 85 - 93.

Dubey, R. S. (1994). Protein synthesis by plants under stressful conditions. In: Hand book of Plant and Crop Stress. Mohammed Pessarakli.(ed.). Marcel Dekker, Inc., New York, pp: 277-299.

Elthon, T. E. and C. R. Stewart (1981). Sub-mitochondrial location and electron transport characteristics of enzymes involved in proline oxidation. Plant Physiol., 67: 780-784.

Evans, L.T., I. F. Wardlaw and R. A. Fischer (1978). Wheat. In: Crop Physiology. L. T. Evans (ed.), pp: 101 - 149. Cambridge University Press, UK.

Food and Agriculture Organisation (1990,1989). Production Yearbook. Rome, Italy.

Gianfagna, T. (1995). Natural and synthetic growth regulators and their use in horticultural and agronomic crops. In: Plant Hormones. P. J. Davis (ed.), pp: 751 - 773. Kluwer Academic Publishers. Netherlands.

Govil, C. M. (1985). Stomatal response to chlorocholine chloride and indol-3-acetic acid in *Commelina communis* L. Proc. Acad. Sci., 95: 167-172.

Grantz, D. A., D. Ho Tuan-Hua., S. J. Uknes, J. M. Cheesman, and J. S. Boyer (1985). Metabolism of abscisic acid in guard cells of *Vicia faba* L. and *Commelina communis* L. Plant Physiol., 78: 51- 56.

Green, C. F., J. D. Ivins, and B. Hunter (1986). Applying chlormequat to *Triticum durum*: response of the crop in the absence of lodging. J. Agric. Sci.(Camb.), 106: 625 - 628.

Gupta, H. S. and D. N. De (1988). Acridine Orange-induced vacuolar uptake of cytoplasmic organelles in plant cells: an ultrastructural study. J. Plant Physiol., 132: 254-256.

Gupta, M. (1992). Effect of growth regulators on foliar stomata of *Vicia faba L.*. Adv. Plant Sci., 5(2): 483-495.

Guttridge, C. G. (1966). The interaction of (2-chloroethyl)trimethylammonium chloride and gibberellic acid in strawberry. Physiologia Plantarum, 19: 397-402.

Halevy, A. H. (1967). Effect of growth retardant on drought resistance and longevity of various plants. Proc. 17th. Hort. Congr., 3: 277-283.

Halevy, A. H., D. R. Dilley and S. H. Wittwer (1966). Senescence inhibition and respiration induced by growth retardants and ^{6}N-benzyladenine. Plant Physiol. 41: 1085-1089.

Halvey, A. H. and B. Kessler (1963). Increased tolerance of bean plants to soil drought by means of growth retarding substances. Nature, 197:310-311.

Hampton, J. G., (1986). The effect of chloromequate chloride application on the seed yield of perennial ryegrass (*Lolium perenne* L.). Journal of Applied Seed Production., 4: 8 - 13.

Hampton, J. G. (1988). Effect of growth retardant and soil residues on succeeding agriculture crops. New Zealand Journal of Experimental Agriculture., 16: 167 - 172.

Handa, S., R. A. Bressan, A. K. Handa, N. C. Carpita and P. M. Hasegawa (1983). Solute contribution to osmotic adjustment in cultured plant cells adapted to water stress. Plant Physiol., 73: 834-843.

Harada, H. and A. Lang (1965). Effects of some (2-chloroethyl)trimethylammonium chloride analogs and other growth retardants on gibberellin biosynthesis in *Fusarium moiliforme*. Plant Physiol., 40: 176-183.

Harker, K. N. and J. S. Taylor (1994). Chlormequat chloride (CCC) pre-treatments may enhance quackgrass *(elytrigia repens)* control with sethoxydim. Weed Technology, 8: (3) 499 - 507.

Harper, F. (1983). Principles of Arable Crop Production. Granada Publishing, London, U.K.

Harris, P. B. (1978). Methods of controlling mildew in spring wheat and the effect of chlormequat and different rates of nitrogen. Exp. Husb., 34: 71 - 82.

Hay, R. K. M. and A. J. Walker (1989). An Introduction to the Physiology of Crop Yield. Longman Group. UK.

Holden, M. (1976). In: Chemistry and Biochemistry of Plant Pigments. T. W. Goodwin (ed.). vol. 2, pp. 1 - 37. Academic Press, London.

Hsaio, T. C. (1973). Plant response to water stress. Ann. Rev. Plant Physiol., 24: 519 - 70.

Hu, C. A., A. J. Delauney and D. P. S. Verma (1992). A bifunctional enzyme (pyrroline-5-carboxylate synthetase) catalyses the first two steps in proline biosynthesis in plants. Proceedings of the National Academy of Science, 89: 9354-8.

Hughes, J. and M. E. McCully (1975). The use of an optical brightener in the study of plant structure. Stain Technology, 50(5): 319-329.

Humphries, E. C. (1963). Effects of (2 Chloroethyl)trimethylammonium chloride on plant growth, leaf area, and net assimilation rate. Annals of Botany, N. S. 27: 107-532.

Jones, M. M. and N. C. Turner (1978). Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol., 61: 122-126.

Jones, R. L., and MacMillan, J. (1984). Gibberellins. In: Advanced Plant Physiology, (eds) Malcolm B. Wilkins. pp: 21-52. Pitman publishing, London.

Kauss, H. (1977). Biochemistry of osmotic regulation. In: Northcote D, (ed.) International Review of Chemistry, vol. II. Plant biochemistry, pp: 119-39.

Kettlewell, P. S., E. A. Whitley and W. S. Meredith (1983). Effects of early application of chlormequat on tillering and yield of winter wheat. J. Agric. Sci., Camb., 100: 735-738.

Kharanyan, N. N. (1972). Effect of retardant chlorocholine chloride on content of protein components in plant leaves during drought. Dokl. Akad. Nauk. SSSR. Ser. Biol., 205: 492-494.

Kuznetsov, E. D., V. F. Vasilenko and V. D. Kreslavsky (1992). Stimulation effects of short-term red light and plant growth retardants on greening and formation on photosynthetic apparatus in wheat seedlings. Plant Physiol. Biochem., 30 (5): 559-564.

Lawlor, D .W. (1995). The effect of water deficit on photosynthesis. In: Environment and Plant Metabolism: Flexibility and Acclimation. N. Smirnoff, (ed) (Oxford:Bios Scientific), pp. 129-160.

Lawlor, D .W. and H. Fock, (1977b). water stress induced changes in the amounts of some photosynthetic assimilation products and respiratory metabolites of sunflower leaves. J. Exp. Bot., 28: 320-328.

Levitt, J. (1980). Response of Plant to Environmental Stress, 2nd. Edition (volume II), Academic Press, Inc. New York.

Loomis, R. S. and D. J. Connor. (1992). Crop Ecology: Productivity and Management in Agriculture Systems. Cambridge University Press, U.K.

Loy, J. B. (1977). Hormonal regulation of cell division in the primary elongating meristems of shoots. In: Mechanisms and Control of Cell Division, (eds) T. L. Rost and E. M. Gifford, pp. 92-110. Dowden, Hutchinson and Ross, Stroudsburg, Pennsylvania.

Ma, B. L. and D. L. Smith (1992). Modification of tiller productivity in spring barely by application of chlormequat or ethephon. Crop Sci., 32: 735-740.

Maksymowych, R. (1973). Analysis of leaf development. Forward by: R. O. Erickson.Cambridge Uni. Press. UK. pp.84-86.

Marcelle, R., H. Clijsters, R. Bronchart and J. M. Michel (1973). Effect of CCC and GA on photosynthesis of primary bean leaves. In: Plant Growth Substances. (ed.) Sumiki, Y., part VII, pp: 1169-1174. Hirokawa publishing Co., Inc. Tokyo.

Matthews, P. R. and J. J. B. Culdicott (1981). The effect of chlormequat chloride formulated with choline chloride on the height and yield of winter wheat. Ann. Appl. Biol., 97: 227 - 236.

Matthews, S., G. O. Koranteng and W. J. Thomson (1981). Tillering and ear production: Opportunity for chemical regulation. In: Opprortunities for Manipulation of Cereal Productivity. A. F. Hawkins and B. Jeffcoat (eds.) Monogr. 7. Br. Plant Growth Regul. Group, Wantage, England pp. 88 - 96.

McCree, K. J. and S. D. Davis (1974). Effect of water stress and temperature on leaf size and on size and number of epidermal cells in grain sorghum. Crop Science, 14: 751 - 755.

McDaniel, R. G. (1982). The physiology of temperature effects. In: Breeding Plants for Less Favourable Environments. Christiansen M. N, Lewis C. F. (eds). John Wiley, New York, pp. 13-45.

Meidner, H., and T. A. Mansfield (1968). Physiology of Stomata. Macgraw-Hill, London.

Meyer, B. S., D. B. Anderson and R. H. % KQL 1960). Enzymes and pigments. In: Introduction to Plant Physiology. Nostrand Company, Inc., Canada, pp. 166-189.

Miflin, R. J. (ed.) (1980). Amino Acid and Derivations, the Biochemistry of Plants, Volume 5, Academic press Inc., New York.

Milthorpe, F. L. and J. Moorby (1979). Photosynthesis and respiration. In: An Introduction to Crop Physiology, pp. 96-131. Cambridge University press, Cambridge, UK.

Miranda-Ham, L, P. Rios and V. Loyola-vargas (1985). Indian journal of Experimental biology. 775: 154.

Naylor, R. E. L., M. E. Saleh and J. M. Farquharson (1986). The response of chlormequat of winter barely growing at different temperatures. Crop Res. (Hortic. Res.), 26: 17-31.

Neale, P. J. and A. Melis (1989). Salinity-stress enhances photoinhibition of in *Chlamydomonas reinhardtii*. J. Plant Physiol., 134: 619-622.

Niemelainen, O. (1987). The effect of some growth regulators on the seed yield of tetraploid red clover. Cited as an abstract in Journal of Applied Seed Production. 5: 67.

Ojeda, H. and S. O. Trione (1994). Effect of (2-chloroethyl)trimethylammonium chloride (CCC) on the growth, biochemistry and rubber yield of guayle plants (*Partenium argentatum* Gray). Journal of Arid Environments, 28: 129-138.

Olumekun, V. O. (1996). An analysis of the response of winter Wheat (Triticum aestivum) components to Cycocel (Chlormequat) application. J. Agronomy and Crop Science, 176: 145-150.

Parkhurst, D. F. and O. L. Louks (1972). Optimal leaf size in relation to environment. J. Ecol., 60: 505.

Pinthus, M. J. (1968). Effects of different applications of CCC (2-chloroethyl)-trimethylammonium chloride on heading and culm characters of wheat. Adv. Frontiers Plant Sciences, 21: 117-126.

Pinthus, M. J. (1973). Lodging in wheat, barely, and oats: the phenomenon, its causes, and preventive measures. Adv. Agron., 25: 209-263.

Plaut, Z. and A. H. Halevy (1966). Regeneration after wilting, growth and yield of wheat plants, as affected by two growth-retarding compounds. Physiologia Plantarum, 19: 1064-72.

Prakash, M., K. Ramachandran and M. Nagarajan (1992). Effect of antitranspirants on leaf temperature transpiration rate and diffusive resistance in Brinjal plants. J. Agronomy and Crop Science, 169: 247-249.

Prasad, M. and R. Prasad (1994). Effect of some plant growth regulators in cotton. Indian J. Plant Physiol., 37(2): 109-110.

Primost, E. (1968). The effect of chlorocholine chloride (CCC) on wheat. Euphytica, 17: 239-249.

Pugnaire, F. I., L. S. Endolz and J. Pardos (1994). Constraints by water stress on plant growth. In: Handbook of Plant and Crop Stress. Mohammed Pessarakli.(ed.), pp: 247-259. Marcel Dekker, Inc., New York.

Purseglove, J.W. (1992). Tropical Crops-Monocotyledons. Longman Scientific & Technical (Fifth impression), Singapore. pp: 291-297.

Quarrie, S. A. and H. G. Jones (1977). Effect of abscisic acid and water stress on development and morphology of wheat. Journal of Experimental Botany, 28: (102) 192 - 203.

Reid, D. M. and A. Crozier (1970). CCC-Induced increase of gibberellins levels in Pea seedlings. Planta (Berl.), 94: 95-106.

Resnik, M. E. (1970). Effects of mannitol and polyethylene glycol on phosphorus uptake by maize plants. Annals of Botany, 34: 497 - 504.

Revilla, M. Eloisa, L. Martin, G. Nicolas, M. E. Legaz and N. Villalobos. (1988). Effects of high temperature on the variation and transport of endogenous cytokinins during the germination of chick-pea seeds. J. Plant Physiol., 132: 223-228.

Richards, D (1980). Scientia Horticulturae, 12: 143 - 152.

Roberts, J. A. and R. Hooley (1988). Plant Growth Regulators. Chapman and Hall, New York.

Robinson, D. R. and C. A. West (1970). Biosynthesis of cyclic diterpenes in extracts from seedlings of *Ricinus communis* L. II. Conversation of geranyl geranyl pyrophosphate into diterpene hydrocarbons and partial purification of the cyclization enzymes. Biochemistry, 9: 80-89.

Rost, F. W. D. (1995). Fluorescence Microscopy. Vol. II. Cambridge University Press. Cambridge, UK.

Rudiger, W. and S. Schoch (1988). Chlorophylls. In: Plant Pigments. Edited by T. W. Goodwin, pp: 1-59 T. J. Press, (Padstow), Cornwall, UK.

Sachs, R. M. and A. M. Kofranek (1963). Comparative cytohistological studies on inhibition and promotion of stem growth in *chrysanthemum morifolium*. American Journal of Botany, 50: 772-779.

Salisbury, E. J. (1927). On the cause and the ecological significance of stomatal frequency with special reference to woodland flora: Philos. Trans. R. Soc. Lond B., 216: 1 - 65.

Salisbury, E. J. (1932). The interrelations of soils, climate and organism, and use of stomatal frequency as an integrating index of the water relation of the plant. Beih Bot. Zool., 99: 402 - 420.

Salisbury, F. B. and C. W. Ross (1985). Plant Physiology (Third Ed.), Wadsworth publishing Co. Belmont California.

Sen, D. N. and S. Mohammed (1994). General aspects of salinity and the biology of saline plants. In: Handbook of Plant and Crop Stress. Mohammed Pessarakli.(ed.). Marcel Dekker, Inc., New York. pp. 125-145.

Shah, C. B. and R. S. Loomis (1965). Ribonucleic acid and protein metabolism in sugar beet during drought. Physiol. Plant, 18: 240-245.

Sheoran, I. S., Y. P. Luthra, M. S. Kuhad and R. Singh (1981). Effect of water stress on some enzymes of nitrogen metabolism in pigeon pea. Phytochemistry, 20(12): 2675-2677.

Simmelsgaard, S. E. (1976). Adaptation to water stress in wheat. Physiol. Plant., 37: 167-1744.

Singh, T. N., D. Aspinall and L. G. Paleg (1973). Stress metabolism. IV. The influence of (2-chloroethyl)trimethylammonium chloride and gibberellic acid on the growth and proline accumulation of wheat plants during water stress. Aust. J. Sci., 26: 77-86.

Singh, T. N., L. G. Paleg and D. Aspinall (1973b). Stress metabolism. I. Nitrogen metabolism and growth in the barely plant during water stress. Aust. J. Biol. Sci., 26: 45-56.

Slatyer, R. O. (1967). Plant Water Relationships. Academic Press, London.

Srivastava, H. S. and R. P. Singh (1987). Role and regulation of L-glutamate dehydrogenase activity in higher plants. Phytochemistry, 26(3): 597-610.

Stalfelt, M. G. (1955). The stomata as a hydrophobic regulators of water deficit of plant. Physiol. Plant, 8: 572-593.

Stewart, G. R. and F. Larher (1980). Accumulation of amino acid and related compounds in relation to environmental stress. In: The Biochemistry of Plants. Vol 5: Amino acids and derivatives B. J. Miflin (ed.), pp: 609 - 635. New York, Academic Press, INC.

Tabora, R. S. and J. G. Hampton (1992). Effects of Cycocel on growth and seed yield of *Lotus uliginosus* Sch. Cv. Grassland Maku. New Zealand Journal of Agricultural Research, 35: 259-268.

Taiz, L. and Zeiger, E. (1991). Gibberellins. In: Plant Physiology, pp: 426-451. The Benjamin/Cummings Publishing. California, USA.

Todd, G. W. and B. Y. Yoo (1964). Enzymatic changes in detached wheat leaves as effected by water stress. Phyton., 21: 61-68.

Tolbert, N. E. (1960). (2-chloroethyl)trimethylammonium chloride and related compounds as plant growth substances. I. Chemical structure and bioassay. Jour. Biol. Chem., 235: 475-479.

Troughton, A. (1980). In: Environment and Root Behavior. D. N. Sen (ed.), pp. 25 - 41. Geobios International, Jodpur, India.

Waddington, S. R. and P. Cartwright (1986). Modification of yield components and stem length in spring barley by the application of growth retardants prior to main shoot stem elongation. J. Agri. Sci., 107: 367 - 375.

Waldren, R. P., I. D. Teare and S. W. Ehler (1974). Changes in free proline concentration in sorghum and soybean plants under field conditions. Crop Sci., 14: 447-450.

Wallace, R. A., G. P. Sanders and R. J. Ferl (1996). Cell structure. In: Biology the Science of Life. 4th (ed.). Harper Collins College Publisher.

Weidner, S. (1987). The effect of the plant growth retardants AMO-16 and CCC on the synthesis of ribonucleic acids and proteins in triticale embryos during the initial phase of germination. Acta Societies Botanicorum poloniae, 56: 303-314.

Wellburn, F. A. M., A. R. Wellburn, J. L. Stoddart and K. J. Teharne (1973). Influence of gibberellic and abscisic acids and the growth retardant, CCC, upon plastid development. Planta, 111: 337-346.

Weyers, J. and H. Meidner (1990). Methods in Stomatal Research. Longman Group, UK. pp: 3-37.

Wiersma, D. W., E.S. Oplinger and S. O. Guy (1986). Environment and cultivar effects on winter wheat response to ethephon plant growth regulator. Agron. J., 78: 761-764.

Wittwer, S. H. and N. E. Tolbert (1960). (2-chloroethyl)trimethyleammonium chloride and related compounds as plant growth substances. V. Growth, Flowering and fruiting responses as related to those induced by auxin and giberellin. Plant Physiol., 35: 871 - 877.

Woolley, E. W. (1982). Performance of current growth regulators in cereals. In: Opportunities for Manipulation of Cereal Productivity. Monograph 7, pp. 44-50. British Plant Growth Regulator Group., pp. 44-50.

Wright, S. T. C. (1969). An increase in the "Inhibitor-β" content of detached wheat leaves following a period of wilting. Planta (Berl.), 86: 10 - 20.

Zagdannska, B. (1984 a). Effect of water stress on CO_2 exchange in flag leaves of spring wheat. Acta Physiol. Plant., 6: 215-221.

Zagdannska, B. (1984 b). Influence of water stress upon photosynthetic carbon metabolism in wheat. J. Plant Physiol., 116: 154-160.