Greening and development in wheat seedlings

Owdah Ayyad Al-Sobhi

IN THE NAME OF ALLAH, MOST GRACIOUS, MOST MERCIFUL

Proclaim ! (or read !) In the name of thy Lord and Cherisher, who created. Created man, out of a (mere) clot of congealed blood. Proclaim ! And thy Lord is most bountiful. He who taught (the use of) the pen. Taught man that which he knew not. (Quran, Iqraa, 1-5).

GREENING AND DEVELOPMENT

IN WHEAT SEEDLINGS.

by

OWDAH AYYAD AL-SOBHI

B.Sc. (King Abdulaziz University)

Kingdom of Saudi Arabia, Jeddah

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DECLARATION

I declare that the work within this thesis submitted by me for the degree of Doctor of Philosophy is my own original work, expect where reference is made to coworkers, and has not been submitted for a degree at this or any other University.

ABSTRACT

The greening of etiolated first leaf blades of wheat (Triticium aestivum Mercia) seedlings (referred to in the text as leaf tissue) was studied in relation to tissue age and water stress. Use was made of whole seedlings, excised leaf blades and leaf blade segments. Responses to photoperiodic illumination were measured as changes in the levels of chlorophyll, total soluble protein and nucleic acids (both total and specific fractions). The pattern of greening in the whole seedlings, excised leaf blades and leaf blade segments was essentially the same in 6 and 10 day-old dark-grown tissues, where chlorophyll accumulation followed the age sequence along the leaf. Least chlorophyll accumulated in the tip of leaves of both ages but the older leaves contained less overall pigment than the younger leaves. Patterns of total soluble protein and total nucleic acid accumulation did not reflect the pattern shown by the chlorophyll. Protein accumulated most in the tip region, with nucleic acids being highest in the middle region.

Water stress treatment reduced chlorophyll accumulation in leaf blade tissue, particularly in the intact seedlings. Protein levels, however, were more variable and appeared to reflect the ability of the younger tissue to accumulate this compound as a stress metabolite. Total nucleic acid levels were also elevated under water stress. Again, these effects were most marked on the intact seedlings, implying that an effect on the roots was also involved. The data from polyacrylamide gel electrophoresis of RNA fractions showed that the level of chloroplast RNA components was maintained up to 17 days for tissue incubated in the dark as well as in the light. Severe water stress treatments applied to the roots of whole seedlings resulted in the loss of ribosomal fraction in the leaves. However, this effect was not seen with mild water stress.

Kinetin treatments during water stress did not appear to alter the pattern

of cell component accumulation although in unstressed material, treatment with this compound enhanced chlorophyll accumulation slightly, especially in the young tissue. This indicates that, at the concentration used, the growth regulator was not able to alleviate the stress condition.

The leaf blades of intact seedlings responded in a specific way which was much more pronounced than for excised leaf blades or leaf blade segments. It was concluded that some signalling was involved between the root and shoot tissues during water stress treatments.

ABBREVIATIONS

ABA	abscisic acid
BSA v	bovine serum albumin fraction v
K	kinetin
cyt	cytoplamic
ch	chloroplastic
CHL	Chlorophyll
ALA	delta aminolevulinic acid
min	minute
h	hour
μm	micrometre
mm	millimetre
cm	centimetre
m	metre
μg	microgram
mg	milligram
g	gram
μl	microlitre
ml	millilitre
l	litre
μmol	micromole
mmol	millimole

mol	mole
μM	micromolar
mM	millimolar
M	molar
°C	degrees Celsius
PEG	Polyethylene glycol
DEP	Diethyl pyrocarbonate
SDS	Sodium dodecyl sulphate
Temed	NNN'N'-tetramethylethylene diamine
TCA	Trichloroacetic acid
Tris	Tris (hydroxymethyl) aminomethane
D.Wt	dry weight
F.Wt	fresh weight
v	Volume
MW	Molecular weight
v/v	volume/volume
w/v	weight/volume
RH	Relative humidity
NA	Nucleic acid
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
mRNA	Messenger RNA
rRNA	Ribosomal RNA
uv	Ultraviolet

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CHAPTER ONE

INTRODUCTION

1.1 General Introduction

In higher plants, the formation of new cells occurs in regions called meristems. The latter can be found at the apex of every root and shoot, and are capable of continual cell division to give indeterminate increases in length (Brown and Broadbent, 1950). The apex of the shoot is enclosed by young leaves which originate from primordia. These are groups of cells a short distance from the apical meristem (Clowes, 1961).

The presence of meristematic regions in the developing primordium has been reported by Esau (1965), Cutter (1964) and Dale (1964, 1982). The formation of new cells in leaves of monocotyledonous plants is from meristematic cells which are located close to the leaf base and surrounded by enveloping leaves (Gifford, 1951, 1963). It has been reported that cell divisions occur throughout the whole of the expanding leaf in dicotyledonous plants (Avery, 1933; Maksymowych, 1963; Saurer and Possingham, 1970; Steer, 1971). In monocotyledonous plants, however, such divisions cease, first in the distal regions and then in the proximal basal parts of the lamina. Langer (1980) reported that in the grass leaf, the cells at the leaf tip become fully differentiated earlier than those at the base. Therefore, the tip of the leaf represents the oldest part and the base the youngest part. Hence, the leaf tip is physiologically mature and is the first part to senesce when the leaf dies.

The photosynthetic abilities of leaf tissues are important in the establishment of seedlings, the growth of plants and the development of fruits and seeds. Correlations of the biochemical and physiological aspects of photosynthesis with morpholog-

ical and ultrastructural features, have been widely investigated (Gunning and Steer, 1975). Particular attention has been given to the development of chloroplast structure and biosynthetic activities during the light-induced greening of etiolated tissues (Armond, 1976). The sequence of events associated with the greening of leaves has been established for a number of species such as bean (Bradbeer et al., 1974). The development of tissue and cell differentiation along the leaf blade are reflected in the varieties of vascular development, biosynthetic capabilities and extent of senescence (Hedley and Stoddart, 1972). The chronology of greening-up of cereal leaves has been reported for a range of species (Grumbach, 1981).

The greening-up and the development of photosynthesis have been studied in leaves under continuous illumination. Very little information is available concerning the effect of light and dark as in natural photoperiods. When light and dark regimes have been imposed, some degradation of chlorophyll has been detected during the dark period (Bennett, 1981). The lability of chlorophyll has been related to turnover of thylakoid protein (Lichtenthaler and Grumbach, 1974). It may, in part, reflect the general turnover of leaf proteins which is influenced by condition of imposed stress (Cooke et al., 1979b). Very little attention has been paid to the development of photosynthetic competence in cereal seedlings, grown under normal photoperiodic illumination, with or without additional physiological stress.

Internal factors, such as the age of tissue and plant growth hormones, are important in controlling plant development and the greening process. Chloroplast development in wheat leaves (as mentioned above) is a linear progression with the youngest chloroplasts in cells near the base and the oldest chloroplasts in cells near the tip of the leaf. Robertson and Laetsch (1974) reported that the age of developing etiolated leaves has a major effect on the rate of chlorophyll synthesis in the presence

of light. Similar results were reported by Biswal and Mohanty (1976) and Biswal and Sharma (1976).

Ageing-induced differential rates of degradation of various leaf pigments such as chlorophyll *a*, chlorophyll *b* and monocarotenoids have also been reported (Biswal and Mohanty, 1976; Whitfield and Rown, 1974). Chlorophyll *a* is more labile during leaf ageing than chlorophyll *b* (Whitfield and Rown, 1974). Misra and Misra (1987) reported that the age of etiolated leaves of barley, bean and wheat has a considerable effect on the greening process. Similar results were reported by Sisler and Klein (1963) and Nadler and Granick (1970). Therefore, the chronological age of leaves is an important factor in the greening process of etiolated tissue.

The effects of plant growth regulators at all levels of development and greening have been reported. Steeves and Sussex (1972) reported that auxins from leaves induce vascular differentiation in the stem. McDavid et al., (1972) showed that auxins induce lateral root production. It has been reported that gibberellic acid prevents the break-down down of chlorophyll (Bata and Neskovic, 1974). Abscisic acid (ABA) affected chloroplast differentiation by reducing the chlorophyll and carotenoids in greening barley seedlings (Railton et al., 1974) and in maize (Mercer and Pughe, 1969). Hayes (1978) and Hammond (1979) found that cytokinins promote leaf unfolding and expansion in intact plants, green etiolated leaf discs and detached cotyledons. Phillips (1975) and Richards (1980) reported that cytokinins promote the growth of lateral buds. Schneider and Legocka (1981) reporte that cytokinins promote chlorophyll synthesis and chloroplast differentiation in plants grown in the dark which are subsequently transferred to the light.

In addition to internal factors, environmental factors play an important role in greening and other developmental processes. The plant is positioned specifically

in relation to light intensity, temperature, nutrients and water supply. Therefore, it is dependent on its environment and exhibits very different behaviour in different environments (Went and Sheps, 1969)

Light has profound effects on the growth process of plants. Bunce et al (1977) reported that the growth of young plant tissue is increased by exposure to light. Schopfer (1972) found that light promoted leaf elongation and the development of proplastids and etioplasts into chloroplasts via its effects on the synthesis of chloroplast constituent molecules such as chlorophyll, ribonucleic acid and protein, (Possingham et al., 1975). Similar results were reported by Kasemir (1983) and Castelfranco and Beale (1983).

The availability of nutrients and water is very important for plant growth (Gifford, 1977). Plants suffering from nitrogen deficiency mature early and the vegetative growth stage is shortened (Mengel and Kirkby, 1978). Nitrogen deficiency is characterized by a poor growth rate; the plants remain small and the stems have a spindly appearance (Hewitt, 1963). Root growth is also affected by nitrogen deficiency and, in particular, branching is restricted (Casper, 1975). Magnesium availability is directly involved in photosynthesis. It has been reported that plants deficient in magnesium have a lower chlorophyll content and a lower photosynthetic capacity (Hewitt, 1963). Ingestad (1972) reported that nitrogen and potassium are required by plants in large quantities. Nitrogen has several effects on plant growth, especially the leaves, such as the expansion of the leaf surface of birch seedlings (Ingestad and Lund, 1979). Robson and Deacon (1978) reported that increased nitrogen levels resulted in faster leaf elongation, greater leaf length and area, and an increased number of tillers in ryegrass. Similar results have been reported by Bhat et al. (1979). In contrast, nitrogen deficiency has been shown to reduce the rate of chlorophyll synthesis (Tevini,

1971a), to lower CO_2 uptake per unit leaf area (Bouma, 1970) and reduce chloroplast size to one half of its normal length (Tevini, 1971a). Potassium is known to be an osmoticum in many physiological processes in plants, such as stomatal opening (Fischer and Hsiao, 1968) and leaf expansion (Mengel and Kirkby, 1980). Marschner and Possingham (1975), working with sugar beet and spinach, reported that leaf cell size increased with increasing levels of potassium.

Water is an essential constituent of all living cells. A plant must maintain its water status near the optimum in order to survive. However, in response to changes in environmental conditions, plants can suffer from either excess of water during flooding or from deficiency of water during water stress (Kluge, 1973).

In recent years, more attention has been paid to biochemical aspects of water stress during plant growth and development. The changes which have been reported in response to water stress depend on the species of plant and on the severity and time course of water stress (Hsiao, 1973; Jones, 1983) Water stress is known to affect many physiological and developmental processes including cell division, cell expansion and primordium development (Slatyer, 1967; Hsaio, 1973). Davies and Van-volkenburgh (1983) and Carmi and Van-Staden (1983) reported that water stress led to a reduction in leaf growth. Mare and Palmer (1976), working with sunflower, found that leaf number can be reduced by water stress. Water stress alters the biological parameters which govern cell enlargement including turgor, wall extensibility and wall yield-stress as reported by Tomos (1985). Brix (1962) and Boyer (1971) reported a decrease in photosynthesis and transpiration in water-stressed plants. Jackson (1962) reported that the growth of root hairs of Agrostis alba seedlings was completely inhibited by a very low concentration of polyethylene glycol (PEG). He also outlined the effect of water loss on plant tissue:-

"a. reduction in the chemical potential or activity of water.

b. increases in concentration of macromolecules and of solutes of low molecular weight.

c. changes in spatial relations in membrane and organelles due to the reduction in volume.

d. reduction of hydrostatic pressure inside the cells.

e. the effect of mild and moderate water stress is likely to be a mechanism of transduction".

Virgin (1965) showed the influence of water stress to be greater on the synthesis of protochlorophyll than on the conversion of protochlorophyll to chlorophyll. He concluded that water stress had little effect on chlorophyll production until the protochlorophyll pool had been exhausted. Duysen and Freeman (1974) also noted that under water stress there was no change in the chlorophyll a/b ratio. However, there was a difference in the total chlorophyll content in the early hours of exposure of the tissue to the light and before the chloroplast developed. Taylor and Rowley (1971) using, sorghum, reported a decrease in photosynthesis during water stress. However, there was no change in total chlorophyll $a + b$. Klein and Neuman (1966) reported a correlation between grana formation and rapid chlorophyll synthesis. A similar result was noted by Boasson et al. (1972). Freeman and Duysen (1975) reported the disappearance of cytoplasmic ribosomes under water stress. Similar results were reported by Mittelheusor and Van Steveninck (1971a).

Cell division and cell enlargement are very sensitive to water stress and are affected in the early stage of plant development (Sivakumar and Shaw, 1978). Mare and Palmer (1976), using sunflower, found that under water stress the number of leaves in the primary stems declined. Troughton and Slatyer (1969) and Slatyer (1969)

concluded that this was a result of stomatal closure. This caused leaf temperature to rise with water stress having an indirect result on photosynthesis. It has been reported that as water stress increases, photosynthesis declines (Boyer, 1970).

Significant differences have been found in the protein levels of plants grown with and without water stress (Bewley, 1981). These depend on the ability of different species and varieties to respond to drought stress. Huffaker (1982) suggested that leaf age and environmental conditions influences protein levels. Dungey and Davies (1982), using barley leaves, found that the rate of protein synthesis in the youngest regions of the tissue was higher than in the older ones. Similar results were reported by Mae et al. (1983). They found that, in a fully expanded rice leaf, ribulose biphosphate carboxylase (RuBisCo) accounted for half RuBisCo accounted for half or more of the total soluble protein content and that, even in the same leaf the protein content of each region was different. Bryant and Fowden (1958) found that leaf age did not affect protein composition in daffodil leaves. However, Viana and Metivier (1980) reported that the total soluble protein changed during leaf ontogeny in Stevia rebaudiana. Bjurman (1959) and Tal (1977) reported that chromosomal genome duplication and physiological activities had a major effect on protein content. Tal (1977), using tomato leaves, found that the protein content was higher in diploid plants than in autotetraploid plants. In contrast, Leech et al., (1985) using leaves of Triticum found that the amount of RuBisCo in a cell was higher in hexaploids than in tetraploids. Diploids contained the lowest amount of RuBisCo. However, Timko et al. (1980) using leaves of Ricinus communis, reported no differences in protein level between haploid, diploid and tetraploid plants.

There are many reports of protein breakdown accompanying water stress. Mothes (1928), Petrie and Wood (1938) and Wilson (1968) found increased protein

breakdown and accelerated leaf senescence in maize plants with increased water stress. Sullivan and Levitt (1959) noted that soluble nitrogen increased and protein declined in the top half of excised succulent leaves during water stress . In addition, nitrogen and soluble nitrogen accumulated in the base of the leaves. Decreased protein synthesis in leaves (Dasgupta and Bewley, 1984), decreased CO_2 fixation (Kramer, 1969; Johnson and Moss, 1976) and a decline in total chlorophyll (Kushnirenko et al., 1976), have also been noted in response to water stress. Levitt (1980) reported that the growth of immature leaves and buds, in the creosote bush, continued even as old leaves died, with severe water stress. The protein content was higher in the younger leaves of creosote than the older ones. In oil seed rape plants, the old leaves wilted faster than the youngest ones (Levitt, 1980). Petrie and Wood (1938), using leaves of the grasses Phalaris tuberosa and Lolium multiflorum, found a decline in protein content and an increase in amino acids during the wilting process. Similar results were reported by West (1962) working with corn seedlings who found that protein levels declined under water stress. In contrast, Chen et al. (1964) found an increase in protein level in citrus seedlings, under extreme water stress. Brady (1973) reported that the synthesis of starch, nucleic acids and protein was inhibited in water-stressed tissues. Gates (1968), working with apical leaves of Lupinus albus, found no loss of proteins under water stress. Shah and Loomis (1965), using sugar beet leaves, found both soluble and total protein content declined during water stress. Similar results have been reported by Stutte and Todd (1969) using wheat leaves and Shiralipour and West (1984a) using maize seedlings. Stutte and Todd (1967) also reported that the amount of protein was higher in drought-resistant wheat varieties than in non-resistant varieties. The response of individual cells in tissues was influenced by changes in other tissues within the plant, for example the root system. Given these observations it is reasonable to assume that cells within a leaf do not

necessarily respond as individuals to water stress, but in relation to the response of other cells.

One of the most common responses of a plant cell to shortage of water is an incease in RNase activity (Diener, 1961; Dove, 1967, 1971; Todd, 1979). Dove (1967) and Kessler (1961) have reported an increase in ribonuclease activity in water-stressed leaves. Arade and Richmond (1976) reported that increases in RNase activity parallel increased water stress, due to an increase in the concentration of abscisic acid (ABA). The stress produced by a medium of high osmotic potential, is a major cause of increased RNase activity. Premecz et al. (1977), using different enzyme inhibitors, concluded that the increase in RNase level is due to enzyme synthesis. Morilla et al. (1973) and Dhindsa and Bewley (1976), working with corn (Zea mays) leaves and the moss Tortula muralis respectively, found that the dehydration of polyribosomes was followed by a decrease in RNase activity. Cocucci et al. (1976) noted a decline in the polyribosomes of water-stressed squash fruit. In addition to the decline in polyribosomes, osmotic dehydration occurred in 0.6 M polyethylene glycol (PEG) reducing the binding of ribosomes to the endoplasmic reticulum in barley aleurone cells (Armstrong and Jones, 1973). The disturbance in protein metabolism caused by water stress depends on a preceding disturbance in nucleic acid metabolism. Levitt (1980) made the following observations on nucleic acids:

1. Nucleic acid (NA) breakdown: the increase in activity of RNase, induced by dehydration, led to a decline in RNA content and protein synthesis. The parts most affected by osmotic stress were the chloroplast components.
2. Nucleic acid synthesis was inhibited by water stress, with decreased incorporation of ^{32}P into some specific fractions in the desert plant Anastatica hierochuntica.
3. Decreased polyribosome content: the greatest affect of water stress on NA compo-

nents was a decline in polyribosomes, such as the disappearance of polyribosomes in maize and bean plants under water stress.

Levitt concluded that RNase destroyed mRNA which binds ribosomes into polyribosomes. Similarly, Chen et al. (1968) showed a decrease in mRNA activity and arrest protein synthesis in wheat embryos under water stress. Hsiao (1970), using Zea mays, showed a shift in polyribosomes to the monomeric form during water stress. Rhodes and Matsuda (1976) found that polyribosomes, in pumpkin seeds, declined under water stress. Similar results were obtained with shoot tissues of peas, wheat and barley.

According to Itai and Benzioni (1973), plant growth regulators are part of the mechanism controlling a plants response to water stress. They supported this as follows:

"1. Water stress evokes concurrent responses in different plant organs which are not directly exposed to the stress. This was found in wheat plants under water stress or when part of the root was exposed to low osmotic potential.

2. The ratio between root and shoot increases owing to reduced shoot growth when plant shoots are exposed to atmospheric stress or roots are exposed to low water potential in their medium.

3. Many developmental changes accompany water stress such as a shortening of life cycle, abscission, dormancy and induction of flowering. These are regulated by a control mechanism of which plant hormones constitute an important part.

4. Moderate water deficits affecting only minute changes in the osmotic potential of plant tissue can cause considerable metabolic changes.

5. Plant response to renewal of the water supply after the stress is characterized by 'overshoot' and the so-called after-effects such as growth rate. Rewatered,

stressed plants exhibit a growth rate surpassing that of the controls. The 'after-effect' is the delay in stomatal opening after turgor is regained by water-stressed plants. These phenomena indicate the existence of oscillation and a 'memory', both features of control systems.

6. Plant responses to different stresses such as heat, low temperature, water and salinity . This may indicate some common regulatory mechanism.

7. The intensity of plant response to water stress varies with plant age and growth condition. These differences indicate the possibility that endogenous hormone levels, which are influenced by plant age and its growth conditions, act as modulators of plant response".

In an attempt to explain changes in protein levels and other components during water stress, a number of workers have looked at coincident changes in plant growth regulators. Itai and Vaadia (1971) found a correlation between the decline of cytokinin in the roots and a decrease in protein synthesis in the leaves. Similarly, it has been reported by Wright and Hiron (1969) that water stress results in an increase in the ABA content in wheat leaves and other species. Mizrahi et al. (1970) and Brady et al. (1979) reported that, during water stress, the effects of ABA are similar to those occurring during natural senescence. All such factors can imitate effects on leaf protein synthesis. It has been reported that senescence and water stress decrease the relative rate of protein synthesis and, under moderate water stress, cytoplasmic polyribosomes decline faster than chloroplast polyribosomes.

Cytokinins have been found to influence stomatal behaviour (Jewer and Incoll, 1981; Blackman and Davies, 1984a). It has been reported that the accumulation of ABA can be stimulated by salt stress (Walker and Dumbroff, 1981), waterlogging (Mizrahi et al., 1972), high and low temperature, (Chen et al., 1983), nutrient defi-

ciency (Goldbach et al., 1975) and by pathogen attack (Ayres, 1981). Under normal water regimes, ABA has been found in mesophyll chloroplasts (Loveys, 1977; Heilmann et al., 1980). According to Hartung et al. (1981) synthesis takes place in the cytoplasm of the leaf mesophyll. Water stress caused a reduction in stomatal pH and then a release of ABA from the chloroplast (Hartung et al., 1981 and Cowan et al., 1982). Most of the growth regulators have been shown to have effects at the cellular level (Cleland, 1986) reducing cell wall extensibility (Van Volkenburgh and Davies, 1983) and hydraulic conductivity (Boyer and Wu, 1978; Cosgrave and Cleland, 1983; Eamus and Tomos, 1983) and affecting cell osmotic properties (Karmoker and Van Steveninck, 1979). Such cellular changes have also been found to be influenced by water stress (Barlow, 1986).

Cytokinin, usually interacting with other growth hormones, can influence different aspects of shoot physiology such as stomatal behaviour, leaf growth, senescence and ABA production. Mansfield and Davies (1985) reported changes in stomata and other drought-related responses to cytokinin and auxin. All these factors can influence water use by the plant (Davies et al., 1986). There are many factors influencing cytokinin transport from roots such as flooding (Burrows and Carr, 1969), salinity (Walker and Dumbuff, 1981) and low temperature (Steponkus, 1982).

Seedling establishment is probably the most critical stage in the life cycle of a plant. Any factors which influence this will eventually have pronounced effects on the growth and development of the mature plant. In this respect, one of the fundamental stages of development is the formation of a photosynthetic system. This involves changes at the cellular and physiological levels in leaves and seedlings as a whole. Chlorophyll production is the most obvious phenomenon seen when seedlings green, but other associated changes in the tissue are also apparent such as protein synthesis.

Any environmental effects, however, will be in relation to the innate changes taking place within the tissue such as ageing. The result is that the effectiveness of external treatment may vary with the age of the tissue.

In order to investigate these aspects of seedling growth, the use of wheat as a model system was employed. This was taken as a representative monocotyledonous species. It was considered suitable since relatively uniform tissue could be grown consistently and quickly. More importantly, because of the nature of the growth of the leaves, it was possible to obtain tissue of different ages within differently-aged plants. One of the problems of studying events in seedling establishment, such as greening, is that they occur over a relatively short of period of time. By manipulation of the period of growth prior to exposure to greening conditions, it is possible to extend the developmental stages and, therefore, allow a study of the events involved. The general aim of the work reported here was to investigate some aspects of the greening process in the first leaf blades of wheat seedlings by analysis of tissue components and at the same time to look at the influence of external conditions on the process. The specific aims of the investigation were to try and answer the following questions:-

1. What are the baseline changes in the tissue components during greening and growth of the first leaf of wheat?
2. Given that chlorophyll levels alter during greening, what changes occur in other components such as protein and nucleic acids?
3. Is the capacity to green a function of the individual tissue areas within the leaf or is it a function of the seedling as a whole?
4. Is there a loss of greening capacity with the age of the tissue and the length of dark growth prior to illumination?
5. To what extent does the imposition of water stress on individual leaf tissues and

seedlings as a whole influence greening and associated developmental processes? In particular, does water stress application to the root cause changes in the leaf tissue?

6. Given the potential role of plant growth regulators as modifiers of water stress conditions, to what extent can cytokinin application influence greening and development both under water-stressed and un-stressed conditions?

1.2 MATERIALS AND METHODS

I.2.1 General Materials And Methods

Wheat Triticium aestivum Mercia caryopses were obtained from Tyne Seed Stores Ltd., Newcastle Upon Tyne. Caryopses were soaked for 12 h in tap water at room temperature (21°C). They were then placed either in trays on sheets of paper towel or trays filled with graded horticultural vermiculite. The trays were placed in a dark growth room at 22°C with relative humidity of 90% (RH). The trays were kept in the dark room for varying periods of time (normally 6 or 17 days) from the day of sowing, depending on the required treatment.

Plants grown in the dark on vermiculite were kept for four days from the day of sowing. These plants were used as a source of etiolated wheat seedlings, excised leaves and leaf segments (tip, middle and base) for various treatments. Only leaf blade tissue was used in this study and is referred to as leaf tissue, excised leaf or leaf segments.

1.2.1.1 Plant Treatment

1.2.1.2 Preparation of Solutions.

Kinetin (K) was made up as a 50 ppm stock solution from which the required dilutions were made; these were stored at room temperature (21^0C). Fresh solutions of polyethylene glycol (PEG) (mol.wt. 8,000) with different osmotic concentrations were prepared by dissolving the appropriate amount of PEG in water. The -5 bar solutions contained 125 g l^{-1} and the -10 bar solutions contained 200 g l^{-1} (Resnik, 1970). A Combination of (K) plus PEG was made up as required.

All reagents were purchased from either British Drug House Ltd., Poole,

Dorset, or Sigma Chemical Ltd., Poole, Dorset.

1.1.2.3 Whole Wheat Seedlings

Seedlings were grown for 6 to 10 days in a dark growth room at 22°C then transferred to 500 ml screw-neck jars so that the roots were submerged in solutions of PEG, K or a combinations of both solutions. 12 seedlings were maintained in each jar for each treatment. The seedlings were held in small holes (5 mm diameter) in the cap of the jar (12 holes in each cap, arranged in two rows of six).

1.1.2.4 Excised Leaf Blades

Etiolated wheat leaf blades from 6 and 10 day-old wheat plants were cut above the sheath. A portion (500 mg) was floated in a polyethylene tube (width 90 mm,length 120 mm and height 60 mm) containing 100 ml freshly prepared PEG, K or a combination of both solutions.

1.1.2.5 Wheat Leaf Blade Segments

Etiolated wheat leaves from 10 day-old wheat plants were excised above the sheath and divided into three equally-sized segments (tip, middle and base). A portion (500 mg) from each region was placed in a covered glass Petri-dish (90 mm in diameter) containing 20 ml of PEG, K or a combination of both solutions. All experiments were performed in the dark. All samples were incubated in either a dark growth room at 18°C or under a 16 h light/8 h dark cycle with a light intensity of 200 μ mol m^{-2} s^{-1} at 23-24°C. The samples were kept in dark and light growth rooms for various periods of time.

1.1.2.6 Fresh and Dry Weight

Tissue from all samples was weighed at the beginning of each experiment,

dried to a constant weight at 70°C for 12 h, then reweighed.

For the extraction of cellular components, the complexity of treatments allowed the use of only two replicate samples of plant material. Therefore, full statistical analysis was not possible. The number of plants or weights of tissue used in each replicate is indicated in the specific component extraction sections.

CHAPTER TWO

CHLOROPHYLL

2.1 INTRODUCTION

Chlorophylls are the principal class of pigments responsible for light absorption in photosynthesis and are found in all photosynthetic organisms. At least seven different chlorophyll types which may be distinguished. These are chlorophyll *a*, *b*, *c*, *d*, bacterio-chlorophyll *a*, bacterio-chlorophyll *b* and chlorobium chlorophyll (bacterio-viridin) (Allen 1966).

The most widely distributed of these pigments is chlorophyll *a*, found in all photosynthetic plant cells. Chlorophyll *a* is the only green pigment in some algae, but in many plant cells it is accompanied by either chlorophyll *b* or chlorophyll *c* Aronoff, 1950; Givan and Hartwood, 1976; Hoober, 1984). Chlorophyll *b* functions as an accessory pigment in higher plants, green algae and euglenoids. In these organisms, chlorophyll *b* comprises from 15% to 50% of the the total chlorophyll content. Most algae contain 25% chlorophyll *b*. Chlorophyll *b* is only detected in greening leaves following the formation of chlorophyll *a* (Augustinussen, 1964). Photosynthetically competent, mutant strains of Chlorella spp. (Allen, 1966) and barley (Boardman and Highkin, 1966) which lack chlorophyll *b* are known. Diatoms, dinoflagellates and brown algae contain, in addition to chlorophyll *b*, small amounts of chlorophyll *c*. This pigment transfers energy (absorbed by the major xanthophyll accessory pigments) to chlorophyll *a* (Vernon and Seely, 1966). Chlorophyll *b* and phycobilin pigments (the latter found mainly in blue-green and red algae) have an important collaborative function with chlorophyll *a* in photosynthesis (Givan and Hartwood, 1976).

The majority of angiosperm seedlings which germinate in darkness do not

contain chlorophyll. Plant tissues that contain little or no chlorophyll are referred to as etiolated and are yellow in colour due to the presence of unmasked carotenoids (Smith and Young, 1956).

In contrast to angiosperms, which form chlorophyll only in light, there are classes of plants which form chlorophyll in the dark. Most gymnosperms and lower plants can produce chlorophyll in the dark (Vernon and Seely, 1966). Algae that normally form chlorophyll in the dark can give rise to mutant strains which require light for greening. This phenomenon has been reported in Chlorella spp. (Granick, 1949), Chlamydomonas spp. (Sager, 1961) and Scenedesmus spp. (Senger and Bishop, 1972). Griffiths and Mapleston (1978) reported that protochlorophyllide reductase in tissues can form chlorophyll *a* in total darkness, in etiolated Picea spp. seedlings and wild type Chlamydomonas reinhardtii. The y-1 mutant of Chlamydomonas spp. which had lost its ability to synthesize chlorophyll *a* in the dark retained the light dependent reductase (Kirk and Tilney-Bassett, 1967). Pinus jeffreyi cotyledons formed chlorophyll in the dark when the seeds were germinated at 23°C. However at 10°C, less chlorophyll was formed (Bogorad, 1950). It has been shown that protochlorophyll is the immediate precursor of chlorophyll during the greening process in leaves. Anderson and Boardman (1964) and Virgin (1981) reported that protochlorophyll is the precursor of chlorophyll *a*. Gassman and Bogorad (1967) and Virgin (1981) summarized the formation of chlorophyll in etiolated plants when they are exposed to light:-

1- The small amounts of protochlorophyll always present in etiolated plants, undergo rapid photochemical transformation to chlorophyll *a*. This transformation takes place immediately after the plant is exposed to light.

2- Additional chlorophyll *a* is formed. This is the greening process and is much

slower than the first step.

3- The formation of chlorophyll *b*. The first traces of chlorophyll *b* appear after about 1 h of continuous illumination .

Liro (1908) found that most seeds did not contain protochlorophyll and the formation of this pigment was a result of the germination process, the pigment is detectable only after the appearance of the root. Smith (1950, 1951) reported the production of protochlorophyll during growth of etiolated barley seedlings. Liro (1908) also reported that the rate of accumulation of chlorophyll is slow compared with the rate of transformation of protochlorophyll. After the initial transformation of protochlorophyll, there is a lag period in which little additional chlorophyll is formed (Gassman and Bogorad, 1967). Subsequently, the rate of chlorophyll accelerates rapidly suggesting that an autocatalytic process is involved. This may be due to an accelerating accumulation of photosynthates which contribute to chlorophyll production. The process of chlorophyll formation slows down at the end of autocatalytic phase, until a steady state is reached. The steady state is considered to be a balance between the production and destruction of chlorophyll (Zavalishina, 1951).

The pigment systems in the leaf are responsible for harvesting and converting light energy into chemical energy (Barber, 1983; Thornber et al., 1979). They are located inside chloroplast on the thylakoid membrane. The pigments, including chlorophyll and carotenoids, are bound to membrane proteins. These pigment-protein complexes have been divided into four categories on the basis of their function (Barber, 1983 and Thornber et al., 1979).

1. The photosystem I (PSI) complex comprises about 30% of the total chlorophyll content of the leaf and is responsible for the transfer of light energy to the PSI

reaction centre.

2. The photosystem 2 (PS2) complex comprises up to 20% of the total chlorophyll and is responsible for the transfer of light energy to the centre of PS2.

3. A light-harvesting chlorophyll *a/b* protein complex (LHCP) comprises 40% to 60% of the total chlorophyll (Thornber et al., 1979; Barber, 1983), LHCP is responsible for collecting about one third of chlorophyll *a* and possibly all chlorophyll *b* into complexes which absorb light and transfer the energy to photosystems 1 and 2 (Butler 1978). Bennett (1983) reported that LHCP is encoded in nuclear DNA and synthesized in precursor form on cytoplasmic ribosomes. The pre-LHCPs are then transferred into chloroplasts and become associated with the thylakoid membrane (Schmidt et al., 1981).

4. Chlorophyll-protein complex IV (CPa) comprises 10% of the total chlorophyll of higher plant chloroplasts (Remy et al ., 1977; Wessels and Borchert, 1978; Waldron and Anderson, 1980) and about 30% of the total chlorophyll in barley mutants (Waldron and Anderson, 1980).

Rauzi and Dobrenz (1970) found that chlorophyll *a* was more abundant than chlorophyll *b* in western wheat grass and blue grama. They reported higher concentrations of total chlorophyll in western wheatgrass than in blue grama. However, Holden (1973) reported that the ratio of chlorophyll *a* to chlorophyll *b* was higher in blue wheat grama than in western wheat grass. It has been reported that the ratio of chlorophyll *a* to chlorophyll *b* in LHCP is 1.3 for higher plants (Hiller and Goodchild, 1981). In some marine algae the ratio was also less than 2 (Nakamura et al., 1976). Baker and Leech (1977) and Webber et al. (1984) reported that the ratio of LHCP, PS1 and PS2 complexes remained constant throughout development. The chlorophyll

a/b ratio has been reported to be affected by length of time of illumination (Thorne and Boardman, 1971). It has been reported that the ratio is constant in young green maize leaves (Boffey et al., 1980). However, the chlorophyll *a/b* ratio decreased with age in greening, etiolated leaves (Boffey et al., 1980). Otto and Young (1976) reported that the chlorophyll *a/b* ratio decreased as total chlorophyll levels declined.

The amount of chlorophyll in plants is affected by internal factors including plant age and plant growth regulators, and external factors including light intensity and water supply (Strain et al., 1971). There are many reports of the loss of pigments from leaves during ageing (Panigrahi and Biswal, 1979). Ageing-induced differential rates of degradation of various leaf pigments such as chlorophyll *a*, chlorophyll *b* and carotenoids have been noted (Biswal and Mohanty, 1976). Robertson and Laetsch (1974) found that the age of developing etiolated tissue had a considerable effect on the rate of chlorophyll formation during greening. Similar results have been reported by Harris and Naylor (1968). Virgin (1983) reported that the amount of protochlorophyll is higher in the tissues at the base of primary wheat leaves than in tissues of the tip region.

Plant growth regulators have different effects on the greening process. Banerji and Laloraya (1967) reported that cytokinin enhances the greening of etiolated leaves exposed to light. Similar results were reported by Shlyk (1971). In ageing etiolated seedlings, cytokinin increased the amount of chlorophyll formation. Similarly, Mlodzianowski and Gezela (1974) reported that cytokinin affected greening in cotyledons by increasing ultrastructural differentiation. El Hinnawy (1974) found that cytokinin induced chlorophyll production in root callus of Melilotus alba .

Light is one of the major external factors affecting chlorophyll formation and is the primary component on the greening and development process of etiolated wheat

leaves (Dale and Murray, 1968).

Light induced accumulation of chlorophyll and chlorophyll-protein complexes have been studied extensively by Lichtenthaler (1981) for radish seedlings and by Virgin and Egnëus (1983) for various cereals. Virgin (1956) reported that the stomata of wheat leaves grown in the dark do not respond to light until a certain amount of chlorophyll has accumulated in the leaves. Also, he found a correlation between chlorophyll content and the activity of stomatal opening in light-grown leaves. Similar results have been reported by Lurie (1977), working with etiolated leaves of Vicia faba. He reported that the start of stomatal opening activity in greening Vicia faba leaves coincided with the start of cyclic photophosphorylation. Freudenberyer (1940) found that the stomata in etiolated leaves opened in the dark in CO_2-free air. Plastids in leaves of plants grown in the shade are longer than those in leaves of plants grown in the sun (Björkman et al., 1972; Boardman, 1977b). However, chlorophyll accumulation in the light is greater than in darkness (Bogorad 1950). It has been reported that chlorophyll *a* accumulation in the cotyledons of Scots pine seedlings is much higher in the light than in the darkness (Kasemir and Mohr, 1981).

The phytochrome molecule is a reversible biological switch, activated by light. A number of light-controlled plant responses are reported to be phytochrome mediated (Smith, 1975). It is believed to be one of the internal factors which is stimulated by red and far-red light (Beevers et al., 1970; Smith 1975, 1976). Miller et al. (1979) and Beevers et al. (1970) reported that red light enhanced chlorophyll formation in barley and wheat respectively. In addition, far-red light reversed the effect of red light effect. It has been reported that red light eliminates the lag phase in chlorophyll formation (Virgin, 1972). Phytochrome appears to affect chlorophyll formation by accelerating protochlorophyllide synthesis (Ford and Kasemir, 1980).

It has been suggested that there may be an inter-action between far-red light and protochlorophyllide. Chlorophyllide conversion to chlorophyll is controlled by delta-aminolevulinic acid (ALA) synthesis (Ford and Kasemir, 1980)

It has been reported that phytochrome increases the rate of formation of ALA, the first step in the chlorophyll biosynthesis pathway (Klein et al., 1977; Masoner and Kasemir, 1975). In seedlings of gymnosperms, far-red light increases the rate of chlorophyll synthesis (Virgin, 1972; Frosch and Mohr, 1980). Oelze-Karow and Mohr (1978) reported that far-red light is important in the formation of chlorophyll *b*.

The greening process is affected by water stress in plants (Hsiao, 1973 and Sundquist et et., 1980). The sensitivity of plants to water stress varies according to metabolic processes and plant species. Plants overcome water stress conditions on rehydration and Hsiao (1973) reported that rehydration affects the greening process of etiolated seedlings. Virgin (1965) found that chlorophyll formation diminished under water stress due to the slower formation of precursors for protochlorophyll. Duysen and Freeman (1974), using etiolated wheat leaves, reported that both the number of thylakoid membranes and chlorophyll synthesis declined during water stress. Duysen and Freeman (1975) reported that moderate water stress inhibited the synthesis of chlorophyll and carotenoids in etiolated wheat leaves and also inhibited ALA synthesis and its conversion to chlorophyll *a*. The decrease in chlorophyll accumulation in response to water stress has been reported by Alberte et al. (1975). Similar results have been reported by Nordin (1976) and Virgin (1965) with etiolated wheat leaves. The latter author suggested that the decrease may be due to a decrease in ALA synthesis.

In this chapter, the results are reported of the investigations of the changes in growth patterns and chlorophyll levels of wheat seedlings exposed to light after various periods of growth in the dark. The growth period regimes were imposed in order to give an age range in the whole seedlings. In addition, the effect of the age range within the individual leaves was investigated on the greening process. From this part of the study it was intended that the baseline pattern of greening could be described. For greening, seedlings were exposed to photoperiodic light regimes (16 h) as opposed to continous light as this was felt to mimic a more natural situation. The influence of imposed water stress, by incubation in PEG, on the growth and greening processes was also investigated.

2.2 MATERIALS AND METHODS

2.2.1 Materials

2.2.1.1 Plant material: as described in General Materials and Methods.

2.2.2 Methods

2.2.2.1 Estimation of chlorophyll in wheat leaf segments using acetone

Wheat leaf segments (tip, middle and base segments from seedlings 6 and 10 day-old) were transferred from Petri dishes, dried with a paper towel and placed in test tubes with 10 ml 80% (v/v) acetone. The tubes were incubated at room temperature (21°C) for 12 h. Absorption was recorded at 645 nm and 663 nm using a spectrophotometer (Unicam SP 800). Chlorophyll concentration was calculated using the equation of Holden (1965).

Total chlorophyll mg $l^{-1} = 20.2_{A645} - 8.02_{A663}$

Chlorophyll *a* mg $l^{-1} = 12.7_{A663} - 2.69_{A645}$

Chlorophyll *b* mg $l^{-1} = 22.9_{A645} - 4.68_{A663}$

2.2.2.1.1 Estimation of chlorophyll in wheat leaves using methanol

Wheat leaves (500 mg) were frozen in liquid nitrogen, ground to a pulp using a pestle and mortar then extracted as follows:-

1. Tissue was homogenized in 4.4 ml extraction buffer (100 mM trizma base, 53 mM sodium acetate, 8.8 mM magnesium acetate, pH 7.2) and transferred to a centrifuge tube.

2. A further 4.4 ml of extraction buffer was added to the pestle and mortar and the washings were combined with the homogenate from (1) above.

3. The homogenate was centrifuged at 2000 g for 10 min at room temperature (21°C) in a bench-top centrifuge (MSE).

4. The supernatant from (3) above was decanted into a centrifuge tube containing 1.0 ml 10% (w/v) aqueous trichloroacetic acid (TCA) and incubated in the dark at 4°C for 30 min.

5. The pellet from (3) above, was resuspended in 90% (v/v) methanol (5 ml) in a centrifuge tube and incubated in the dark at 4°C for 1 h.

6. The suspension from (5) above, was centrifuged, the supernatant decanted into a tube and made up to volume (5 ml) with 90% (v/v) methanol.

7. The methanolic extract from (6) above, was scanned in a spectrophotometer (Ultrospec 4050 Biochrom) at 650 nm and 665 nm.

Total chlorophyll was calculated from the equation of Holden (1976).

total chlorophyll mg l^{-1} $=25.5_{A650} + 4.0_{A665}$

Parts of this procedure were similar to those used for protein extraction (see Chapter 3)

2.3 RESULTS

2.3.1 Leaf growth responses

The effect of incubation in the light (16 h photoperiod) on the growth of dark-grown wheat seedlings during greening was determined, as a preliminary investigating cell component changes.

2.3.2 Leaf dimension measurements

Wheat seedlings (6 day-old) were transferred from dark conditions and incubated in the light or kept in the dark with various treatments for 24 h or 72 h, as described in (General Materials and Methods) page 38.

The data in Table 1.1 show that the first leaf of the seedlings grew by a similar amount whether in the light or in the dark, with water. The effect of water stress on growth of the first leaf was tested by incubating seedlings with their roots in a solution of PEG at -5 bar and -10 bar. The growth of first leaves was reduced markedly by the low concentration of PEG in both the light and the dark incubations. There was, however, a very marked reduction in leaf growth with the higher concentration of PEG in the light and dark samples (Table 1.1). It was noted that K slightly reduced the growth of seedling leaves in the light, but it had no effect on seedlings which were incubated in the dark. The effect of K, in combination with PEG, on the growth of leaves was investigated to see if there was a counter effect by the growth regulator. the data in Table 1.1 show that K treatment along with high and low concentrations of PEG caused essentially the same reduction in leaf growth as caused by PEG alone.

The influence of leaf age on growth processes was also investigated using 10 day-old seedlings. Similar results were obtained for 10 day-old seedlings treated as stated above for 6 day-old seedlings. Leaf size increased after 72 h in both light and

Table 1.1
Leaf length increase (mm)

Treatment	Light		Dark	
	24 h	72 h	24 h	72 h
H2O	13.2	31.4	10.4	30.2
K	10.8	27.0	10.8	30.8
-5 PEG	6.4	16.0	2.8	14.6
-10 PEG	1.4	2.8	1.0	1.8
-5 PEG + K	4.4	19.4	4.8	11.8
-10 PEG + K	1.0	2.4	1.4	1.6

Table 1.2
Leaf length increase (mm)

Treatment	Light		Dark	
	24 h	72 h	24 h	72 h
H2O	6.4	12.6	9.2	16.4
K	7.4	8.4	9.0	14.6
-5 PEG	2.0	5.8	5.0	9.0
-10 PEG	1.0	1.2	1.0	1.2
-5 PEG + K	3.4	6.6	5.0	7.6
-10 PEG + K	1.0	1.2	1.2	2.2

dark incubation on water; however, the growth was less than observed with 6 day-old leaf tissue (Table 1.2). This indicates that by 13 days the leaf blade was reaching its maximium growth capacity. K treatment caused a slight reduction in the growth of older seedlings in both light and dark treatments (Table 1.2).

The results of the effect of PEG on the growth of older leaves, when compared with water controls is shown in Table 1.2. There was a reduction in their growth with both low and high concentrations of PEG. However, there was a more marked reduction in leaf growth with high concentration of PEG. This occurred both in light and dark incubations for 24 and 72 h. However, there was a slight increase in the leaf growth with low concentrations of PEG, in the dark and the light, for the period of incubation. The effect of PEG on the leaf growth (as measured in terms of leaf length increase) was more pronounced with older leaves than younger ones.

The effect of a combination of PEG and K was tested on the growth of leaves (Table 1.2). There was no effect of K over PEG on the growth. Therefore, the PEG influence was not countered by the growth regulator.

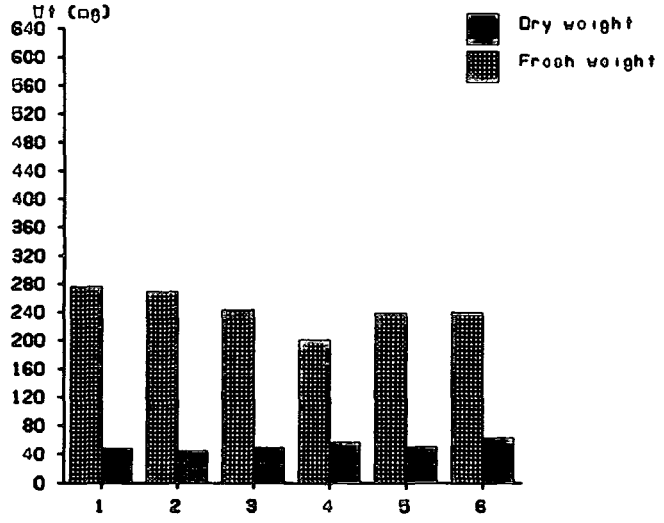
2.3.3 Weight measurements

The effect of water stress on the fresh and dry weight of greening etiolated seedlings was also investigated. Dark-grown wheat seedlings (6 days-old) were incubated in the light and the dark for 48 h and 72 h with their roots in either water or a solution of PEG as described in General Materials and Methods. The data in Fig. 1.1, 1.2, 1.3 and 1.4 show the fresh and dry weight of seedlings, when incubated for 48 h and 72 h in the light and the dark.

The data in Fig. 1.1 show that overall the fresh weight of the roots for seedlings incubated in the dark for 48 h was reduced as opposed to seedlings incubated

Figure 1.1

a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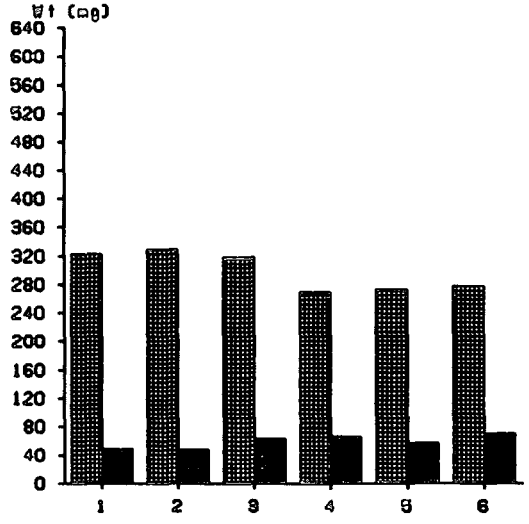
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Figure 1.2

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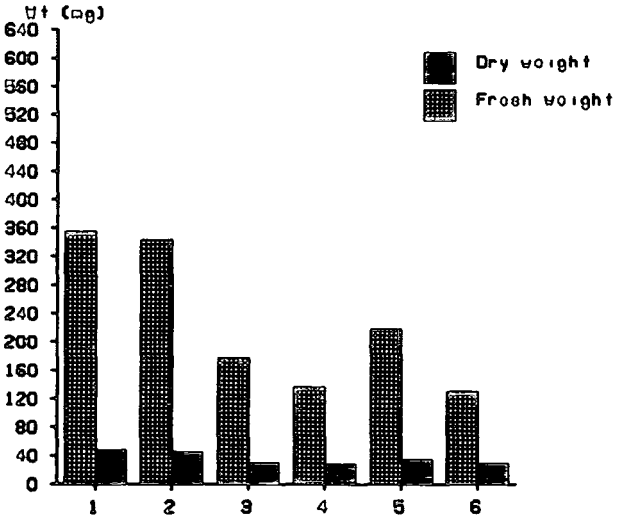
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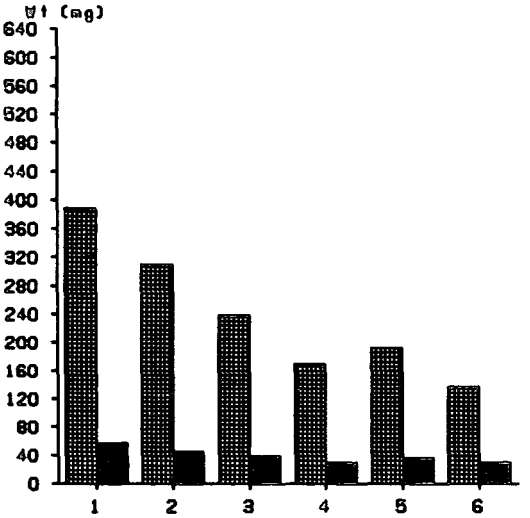
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Figure 1.3

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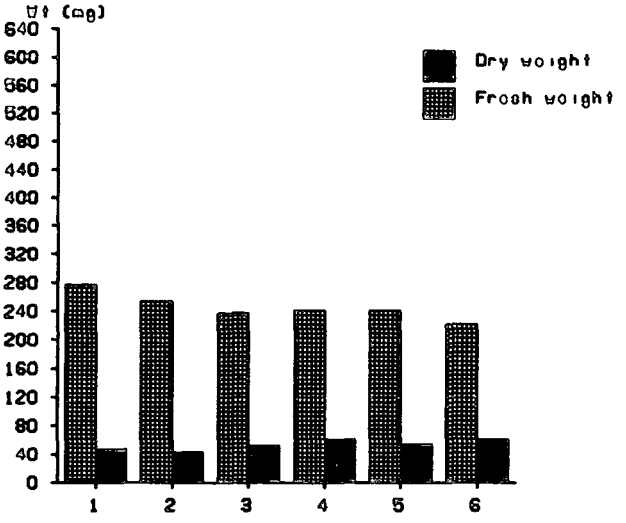
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Dry weight
Fresh weight

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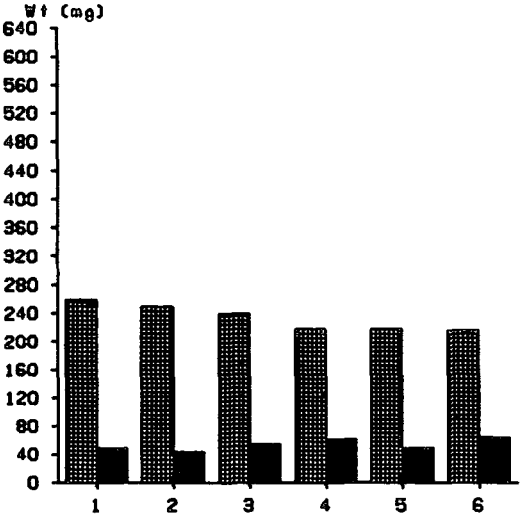
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Figure 1.4

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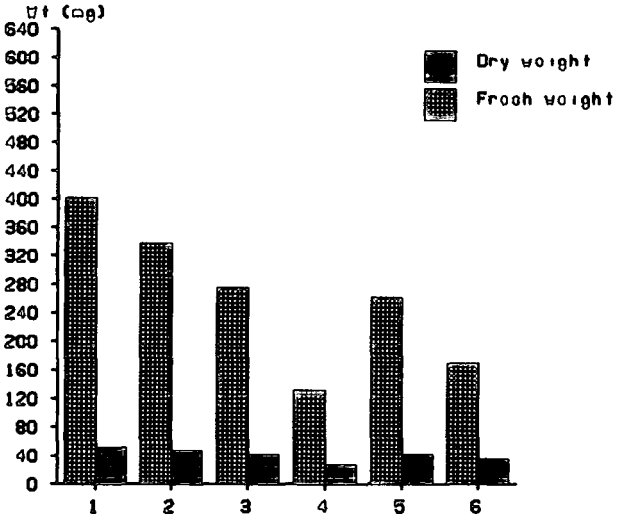
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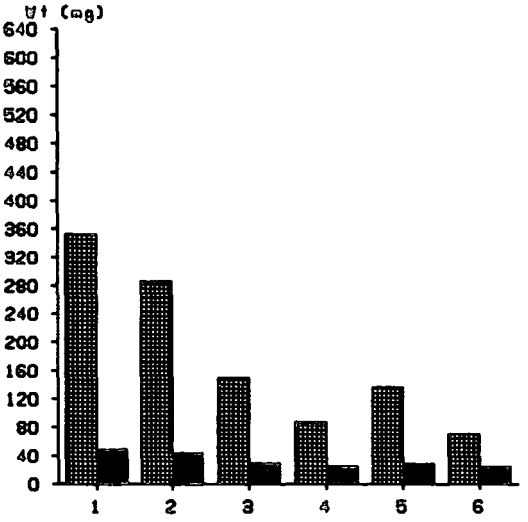
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in the light. No effect of K on root fresh weight was noted in either case. PEG alone appeared to be only slightly effective in reducing the fresh weight of seedlings incubated in the light and the dark and only at the higher concentration. In both light and dark conditions the combinations of PEG and K caused a slight reduction in fresh weight in relation to the water or K. In light-incubated seedlings, the dry weight of the roots was slightly increased with PEG treatments. However, this effect was not as apparent in the dark-incubated seedlings. This pattern of effect for the leaves of the seedlings is shown in Fig. 1.2. There was a pronounced reduction in the fresh weight of the leaf which was much more marked than for the root systems. However, the reduction in the fresh weight of the leaf was more noticeable in the dark-incubated than light-incubated seedlings. PEG caused a reduction in the fresh weight of the leaf in the dark and the light, but the effect was higher in the dark. In contrast, K caused a slight decrease in the fresh weight of the leaves of those plants incubated in the light compared to the plants incubated in the dark. The effect of a combination of K and PEG was found to be similar to the effect of PEG alone in both concentrations(reduction in the fresh weight of the leaf).

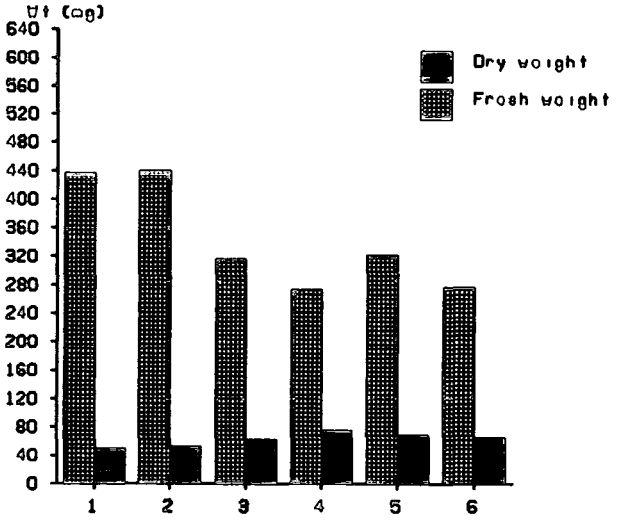
The fresh weight of roots of seedlings which were incubated for 72 h are shown in Fig. 1.3. There was no effect of PEG or K or a combination of them on the fresh weight of the roots of plants incubated either in the dark or in the light. The dry weight of the root was the same with all treatments in both the dark and the light incubation. It was also found that PEG had essentially no effect on the dry weight of roots. However, when the fresh weight was determined for the first leaves after 72 h of incubation, it was noted that the effect of PEG was similar to the 48 h incubation. There was a reduction in leaf fresh weight in both the dark and the light incubated plants (Fig. 1.4). However, the reduction in leaf weight was

greater than for the 48 h treatment. However, the reduction in the fresh weight of leaves after 72 h was higher in plants incubated in the light than in those incubated in the dark. PEG caused a reduction in the fresh weight in both treatments in the dark and the light and the fresh weight was lower with the high PEG concentration than the low PEG concentration. K treatment caused a reduction in the leaf fresh weight in both the dark and the light in relation to the control. Treatment with a combination of K and PEG showed a similar pattern to PEG alone. K did not appear to counter the effect of PEG in the leaf tissue. However, the growth regulator did cause a slight reduction in leaf fresh weight in relation to the water in both the dark and the light-incubated seedlings. Dry weight determinations indicated a different pattern for leaves compared with the roots. The dry weight decreased with all PEG treatments in the leaves in relation to the water control. This effect was seen for seedlings incubated in both the light and the dark for 72 h.

In order to investigate the influence of the age of leaf tissue on growth during greening, 10 day-old wheat seedlings were incubated and treated in the same way as mentioned before for 6 day-old wheat seedlings. PEG caused a reduction in the fresh weight of roots of 10 day-old seedling with both PEG concentrations in the dark and the light after 48 h of incubation (Fig. 1.5). However, the reduction in the fresh weight was greater in the light than in the dark. K did not overcome the effect of PEG in a combination of PEG and K. However, there was very little effect of K alone. It was noted in Fig. 1.5 that the dry weight of root was slightly higher with both PEG treatments or in combination with K. The effect of K treatment on the dry weight of root is shown in Fig. 1.5. K caused a decrease in the dry weight of root in the light in relation to the water or PEG treatments. In the dark incubation, K had no effect on the dry weight of the root and it was the same as control.

Figure 1.5

a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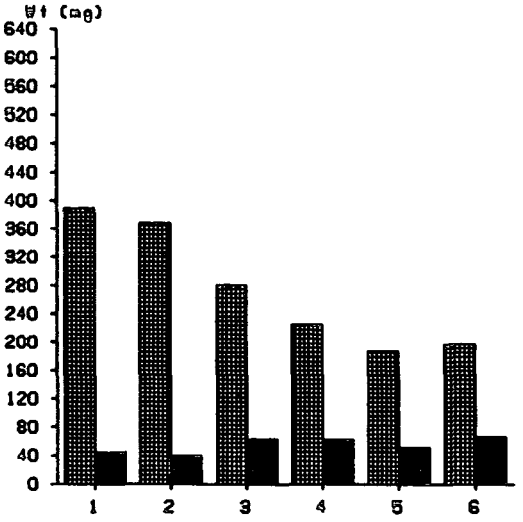
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Figure 1.6

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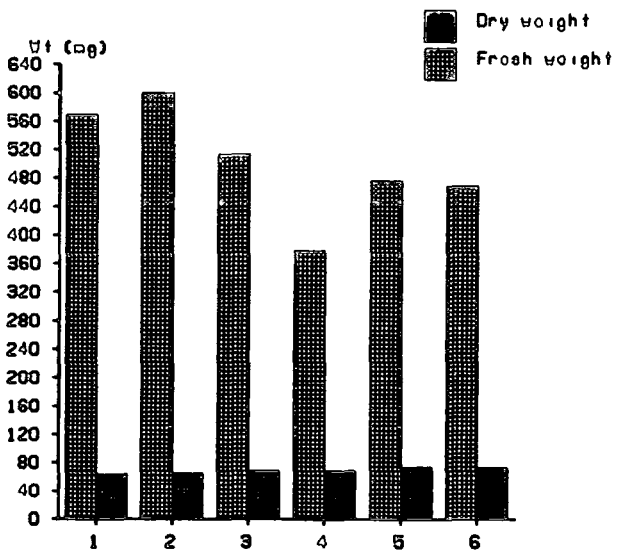
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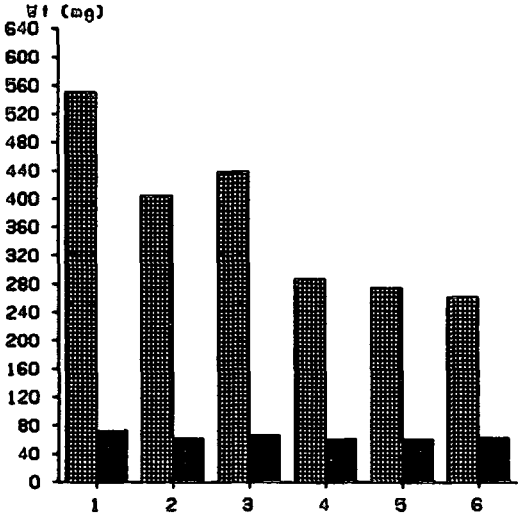
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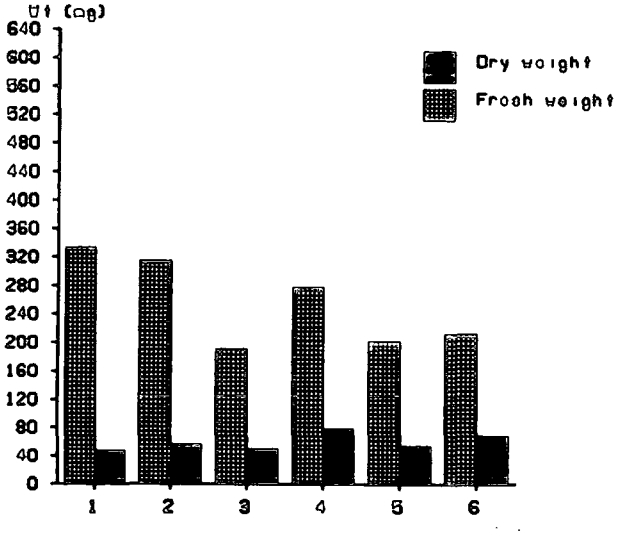
The data in Fig 1.6 show the fresh and the dry weight for leaves of 10 day-old seedling incubated in the dark and the light for 48 h. There was a reduction in the fresh weight of the leaves and the reduction was greater in the light than in the dark. Again, K appeared to have no countering effect on PEG. However, K treatments alone only had an effect in the dark. The dry weight of the leaves was similar in all treatments (Fig. 1.6).

The data in Fig. 1.7 illustrate the effects of PEG, K and a combination of the two compounds on the fresh and dry weights of of roots of 10 day-old wheat seedlings following incubation for 72 h either in the dark or light. The fresh weight of the root was reduced with both PEG concentrations in the dark and the light in relation to water controls. K had no effect on the fresh weight of roots of 10 day-old plants when applied either as a single treatment or in combination with PEG in both the dark and the light. The dry weight of roots was increased by PEG treatment and was the same as for 48 h root treatments (Fig. 1.5). K had no effect on the dry weight of roots of 10 day-old plants in both treatments in the dark and the light. The data in Fig. 1.8 represent the fresh and dry weight determinations for leaves of 10 day-old plants incubated for 72 h in the dark and the light. A more marked reduction in the leaf fresh weight was seen in the light-incubated seedlings compared to those plants incubated in the dark, for the PEG treatments. K treatment reduced the fresh weight of the leaf samples incubated in the light (in relation to the water control) more than in the samples incubated in the dark. A combination of K and PEG resulted in a reduction of the fresh weight of the leaves and no marked countering effect of K was seen (Fig. 1.8). The dry weight of leaves of 10 day-old plants was slightly higher in the light-incubated samples than the dark-incubated samples with PEG, K and a combination of both compounds (Fig. 1.8).

Figure 1.7

a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Dry weight
Fresh weight

b

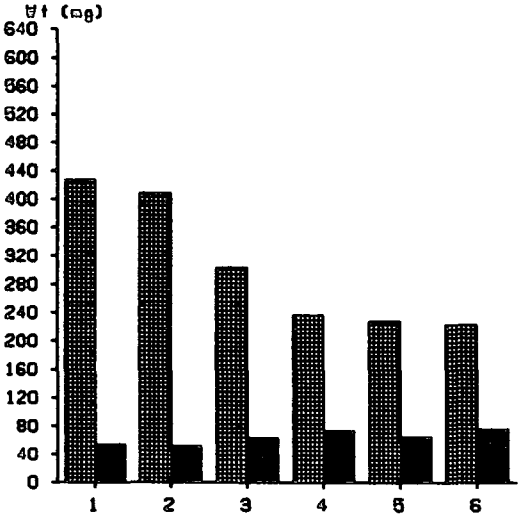
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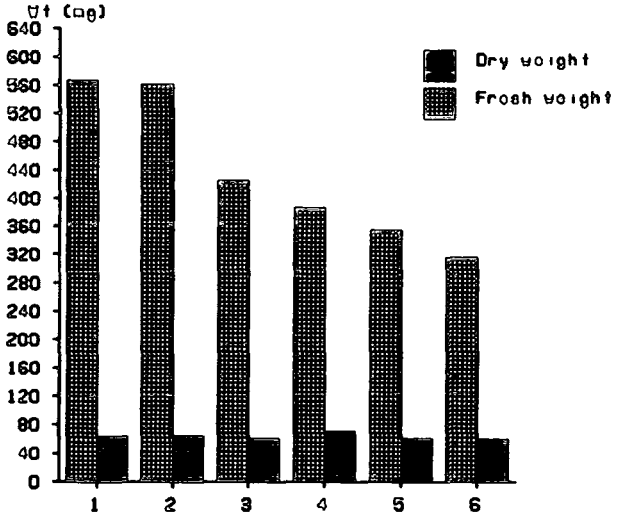
Figure 1.8

a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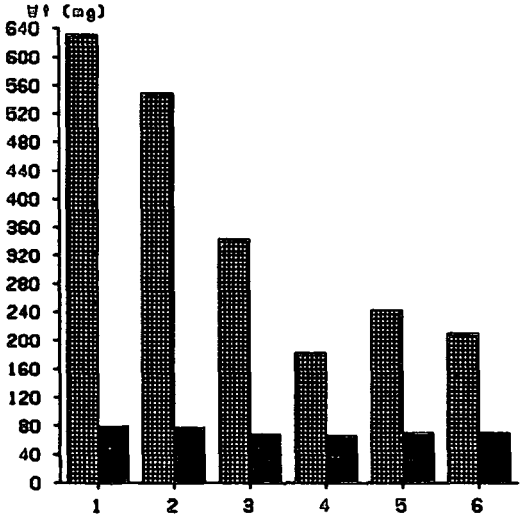
Dry weight
Fresh weight

b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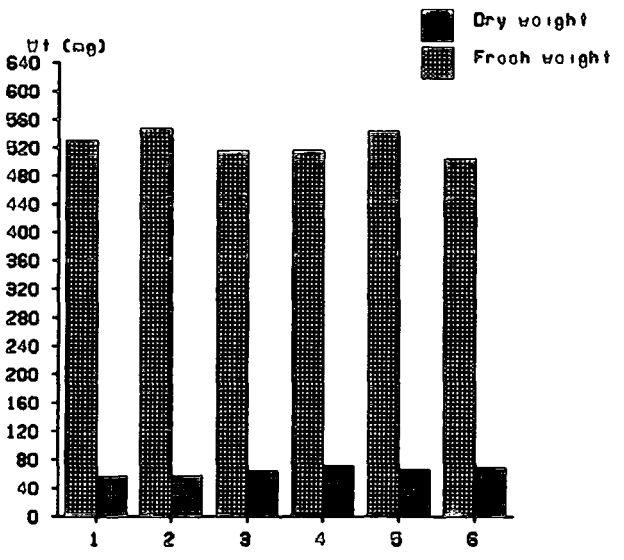
The role of the root in relation to effects on the leaves was investigated using excised leaf and leaf segment tissue. Excised dark-grown leaves (6 and 10 day-old) were incubated both in the light and the dark in Petri-dishes with or without PEG for 48 h and 72 h. Leaf tissue (500 mg) was placed in a Petri-dish with low and high concentrations of PEG solution, K or a combination of PEG and K solution. At the end of the incubation period, the tissue was re-weighed and placed in an oven for 12 h at 70°C and dried to a constant dry weight. Fig. 1.9 and 1.10 show the fresh and the dry weight determinations for the 6 day-old excised leaves incubated in the dark and the light for 48 h and 72 h.

The fresh and the dry weights were essentially the same in all treatments with a solution of PEG (-5 bar, -10 bar) or K or a combination of PEG and K. Fresh and dry weights were the same as the water incubated control samples. However, the fresh weight of the tissue increased slightly from the original weight (500 mg) in all treatments. For 10 day-old excised leaves treated and incubated as above, there was a similar pattern for fresh and dry weights (Fig. 1.11 and 1.12). It appears that the age of the tissue did not alter the response substantially.

In order to investigate the effect of PEG or K or a combination of both solutions on leaf sections, leaf segments were incubated as for excised leaves. Dark-grown leaves (10 day-old) were segmented into 3 segments (tip, middle and base) and incubated as stated before for 48 h and 72 h in the dark and the light. The data in Fig. 1.13 and 1.14 show the fresh and the dry weight of the leaf segments (10 day-old) incubated in the dark and the light, respectively. There was a slight effect of PEG at low and high concentrations, reducing the fresh weight of the leaf segments in both the dark and the light. It was more noticeable in the light than the dark. K did not effect the fresh weight of the segments in either case. However, a combination of K

Figure 1.9

a


Dry weight
Fresh weight
Wt (mg)
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b

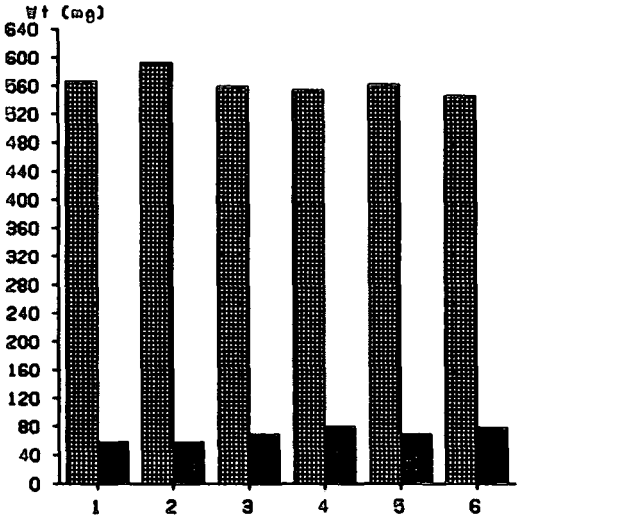
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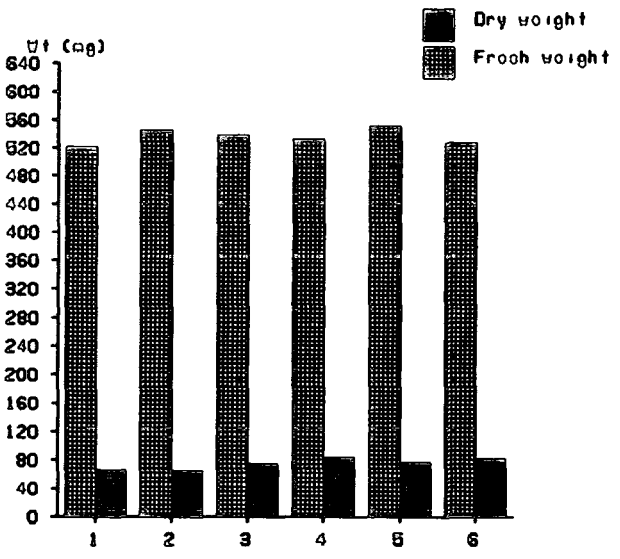
Figure 1.10

a



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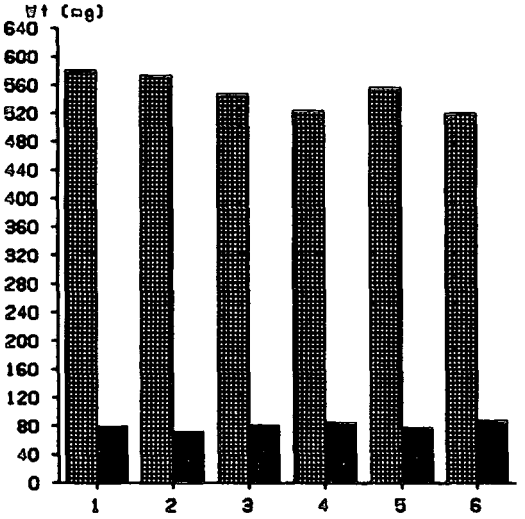
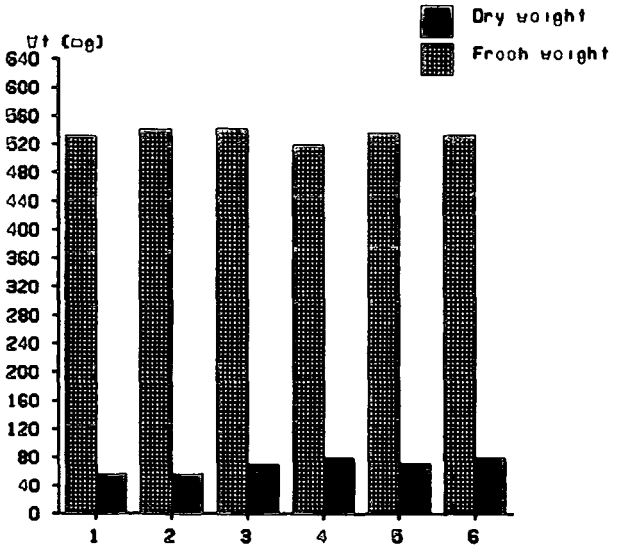


Figure 1.11

a



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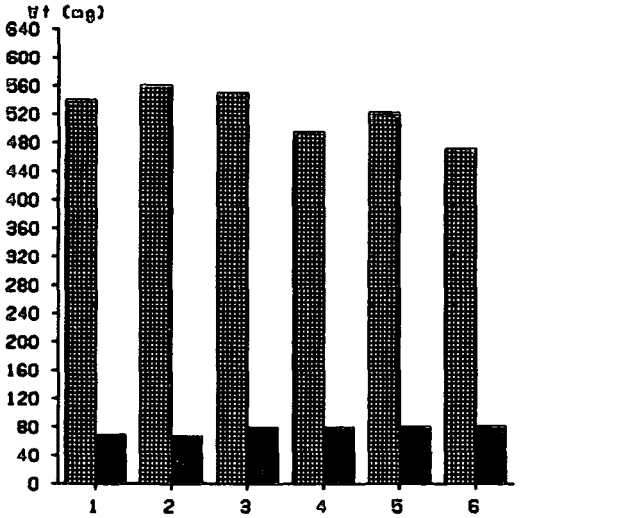
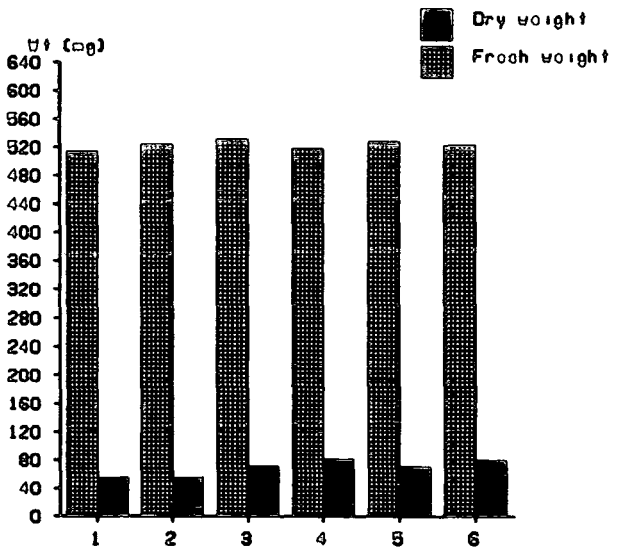


Figure 1.12

a



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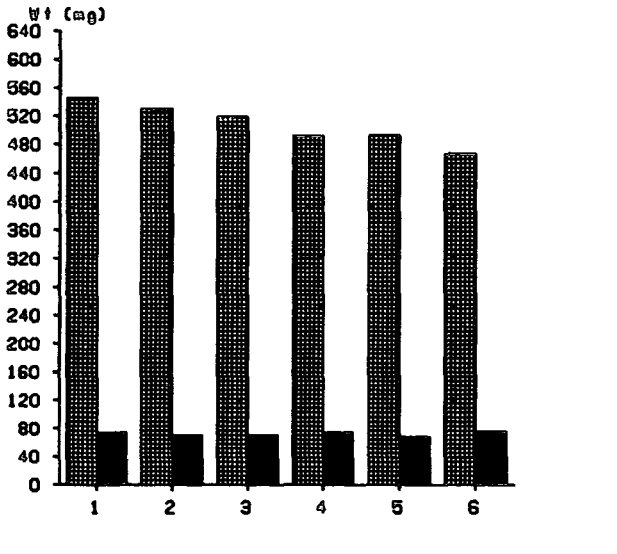
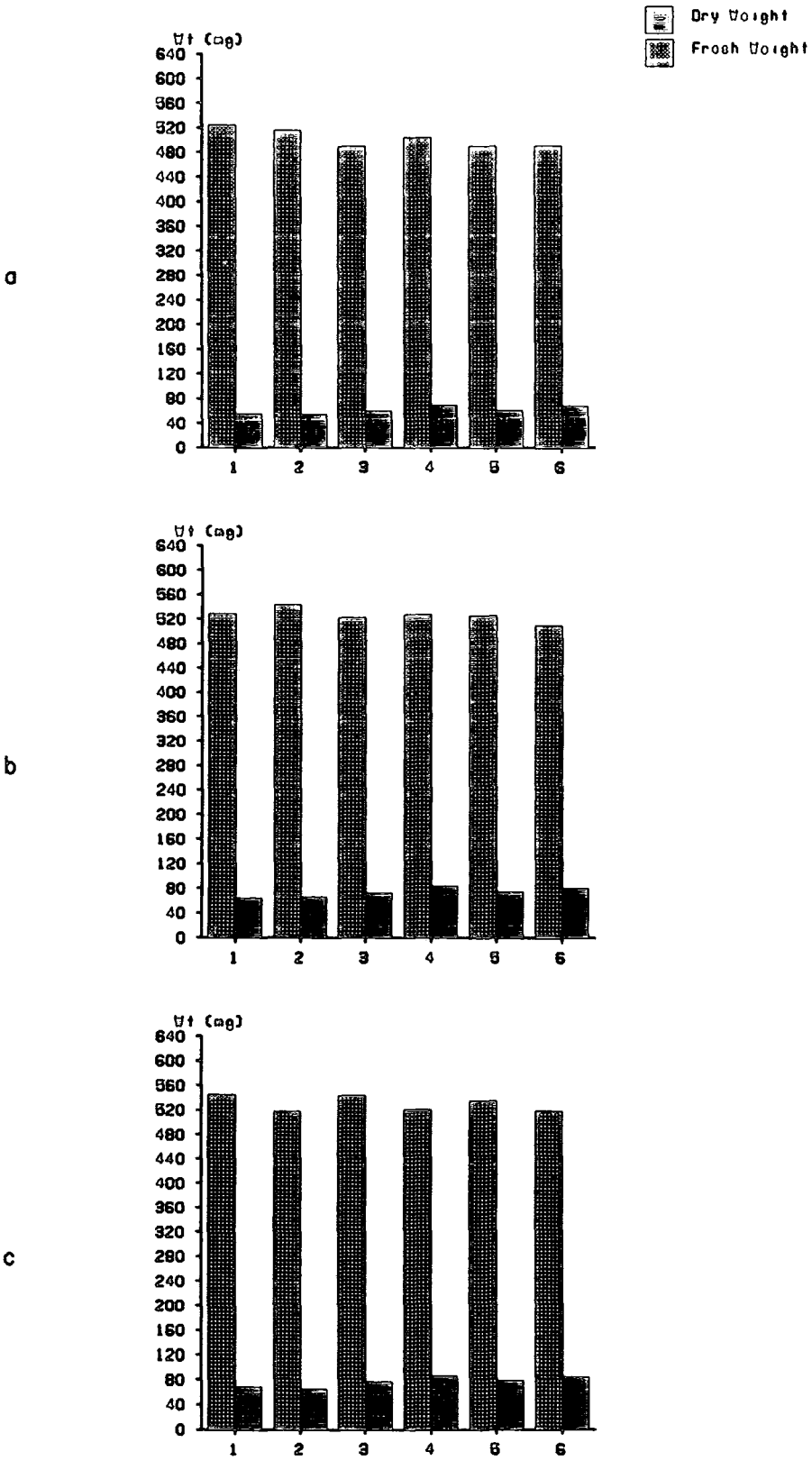


Figure 1.13


Dry Weight
Fresh Weight
Wt (mg)
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6
a
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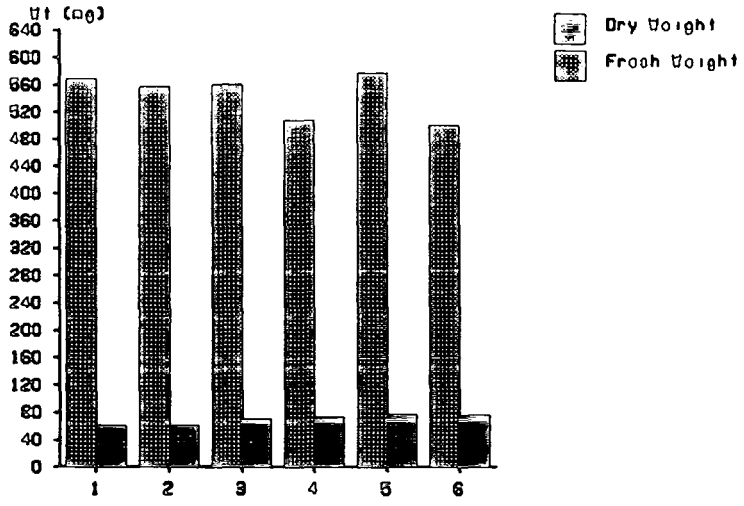
c

Figure 1.14

a



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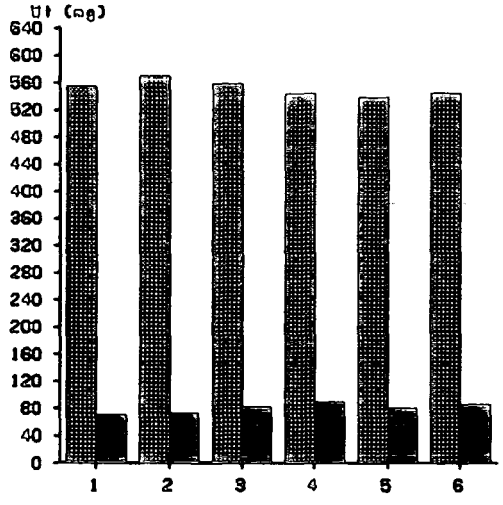

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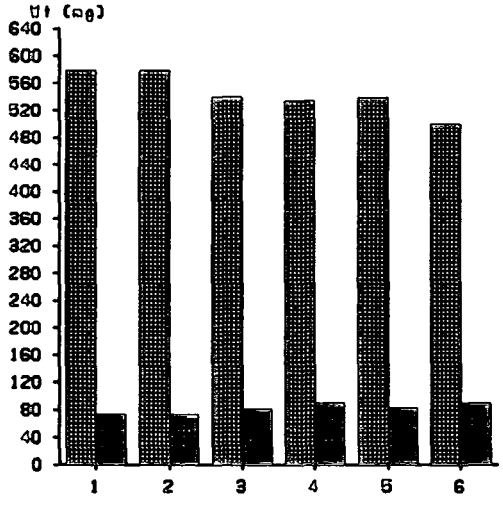
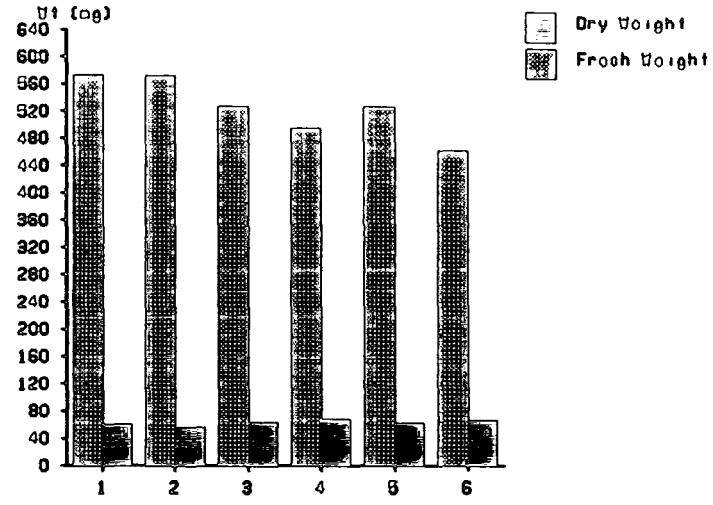


Figure 1.15

a



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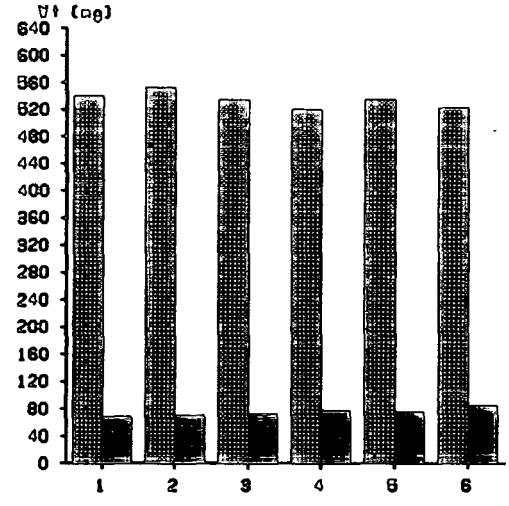

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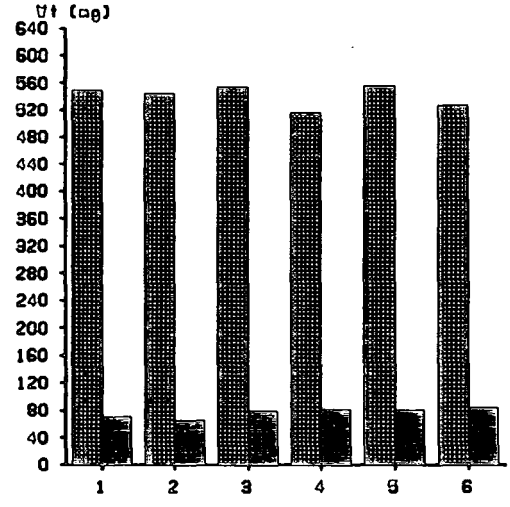
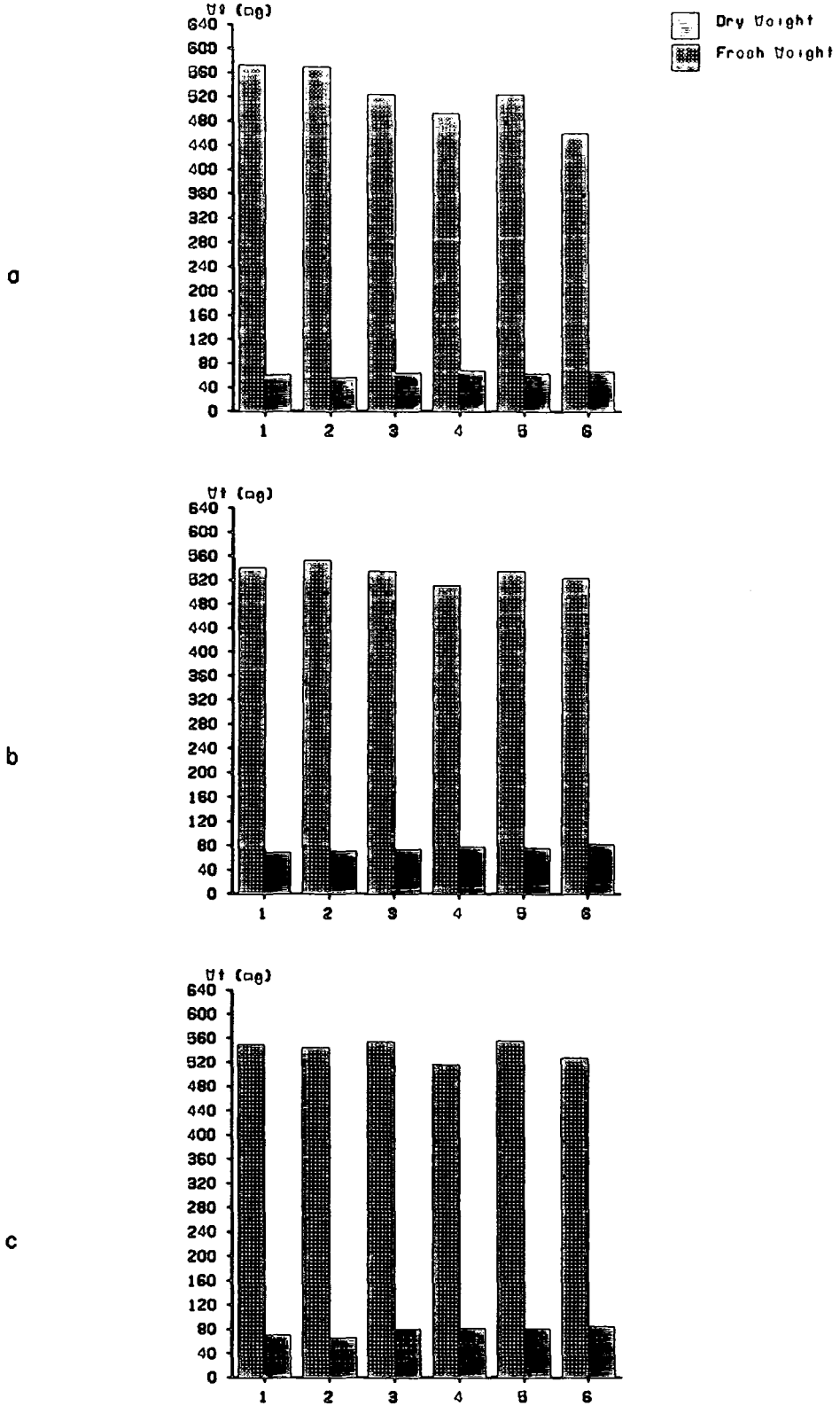


Figure 1.16


Dry Weight
Fresh Weight
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and PEG gave a similar pattern as PEG alone.

The results for leaf segments incubated in the same way for 72 h in the dark and the light are shown in Fig. 1.15 and 1.16. The effect of PEG at low and high concentrations was more effective in reducing the fresh weight of the leaf tip segments than other segments in both the dark and the light. K treatment did not effect the fresh weight of the segments. However, the effect of a combination of K and PEG on the fresh weight of the leaf segments was similar to that at 48 h. The data in Fig. 1.13, 1.14, 1.15 and 1.16 show that the dry weight for all segments in the dark and the light was similar for the leaf segments treated and incubated for the same period of time. However, PEG, K or a combination of K and PEG appeared directly to affect the root system, not the excised leaves or the leaf segments.

2.4 Leaf greening responses

Chlorophyll accumulated by the first leaves of dark-grown wheat seedlings was investigated in relation to the age of the plants and the age within the leaf.

2.4.1 Effect of age of leaf on the greening process.

Wheat seedlings (10 day-old) were exposed to 72 h of light following growth in the dark for 10 days (Plate 1.1). It was noted that the greening occurred along the leaves, but was not equal. It was noted that the leaf tip still did not green fully over this period of time. It appeared, therefore, that the capacity to accumulate chlorophyll was lost in the older tissue.

The first leaves of seedlings, treated as above, were divided into three equally-sized segments and the chlorophyll extracted as described in General Materials and Methods. The data in Fig. 1.17 show the total extracted chlorophyll levels of the tip, middle and basal segments. It can be seen that the segments differed in their ability to accumulate chlorophyll. The level of chlorophyll was higher in basal segments, followed by the middle segments. Least chlorophyll was found to accumulate in the leaf tips. Therefore, the sequence of greening potential down the leaf and the capacity to green were not equal along the length of the leaf. This pattern followed the age sequence down the leaf where the tip contained the oldest cells and the base of the leaf contained the youngest ones. This differential capacity for greening could have been a function of tissue interaction along the leaf and in order to test this, use was made of excised leaf segments comparable to the areas of segmented leaves.

Three equally-sized leaf segments were cut from the first leaves of wheat seedlings which had been grown in the dark for 9, 11, 15, 17 or 20 days. These segments were incubated on water in Petri-dishes for 24 h or 48 h in the light growth

Plate 1.1

The pattern of greening of wheat seedlings grown in the dark for 10 days then incubated in the light for 72 h.

Figure 1.17

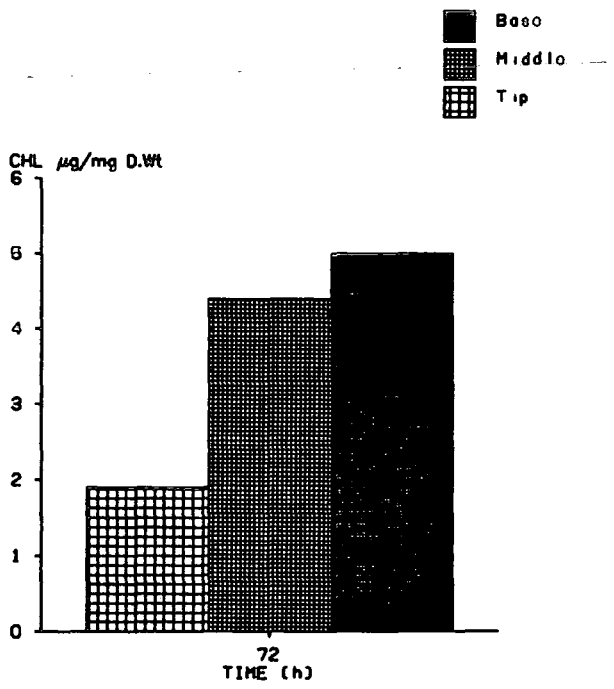
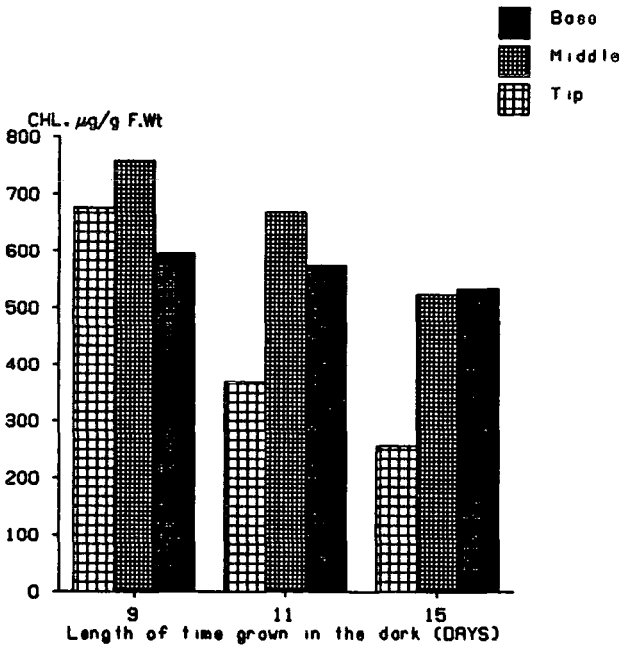


Figure 1.18

a



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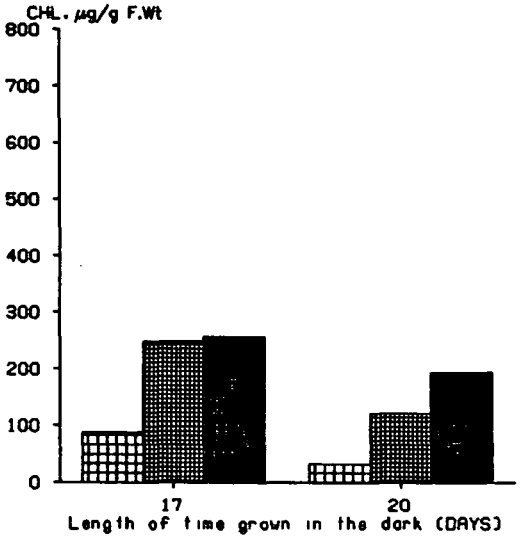


room. The data in Fig. 1.18 show that older leaf tissue (tip) was less able to accumulate chlorophyll than the other two areas after 11 and 15 days growth in the dark followed by illumination for 24 h or 17 and 20 days dark growth followed by 48 h of illumination. However, the pattern of greening of tip, middle and base segments was maintained with segments in isolation from one other, giving essentially the same relative accumulation of chlorophyll as in the intact seedlings.

It appears that the pattern of greening along the leaf is maintained after segmentation, prior to illumination. The possibility that the greening pattern originally observed in the intact seedling was due to interaction within the leaf tissue does not therefore seem to be the case. The influence of age on this greening process is apparent since the tip region initially has a relatively high capacity for chlorophyll accumulation after 11 days dark growth but this is lost gradually up to 20 days. A similar pattern is seen for the middle segment. However, whilst the total capacity for chlorophyll accumulation of the basal segment declined with time, accumulation relative to the other segments actually increased up to 20 days.

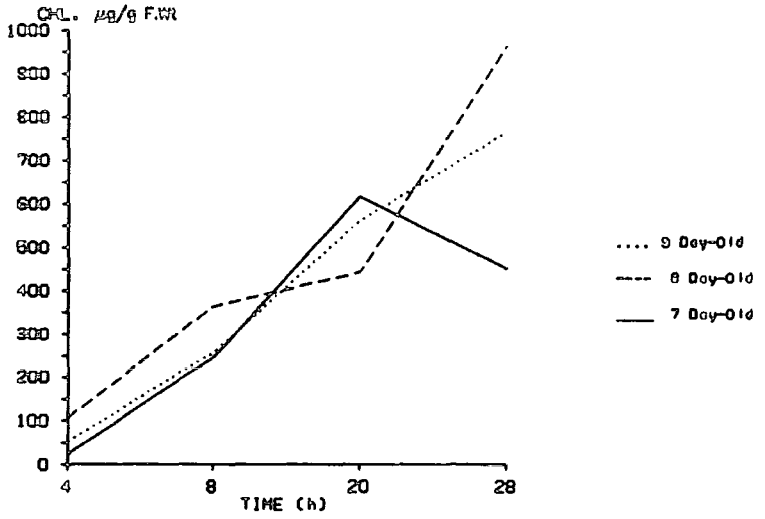
The observed pattern of greening, therefore, appears to be related to the tissue age and hence to cell age. It should be noted that even after 20 days of incubation in the dark wheat tissue still retained some capacity for the accumulation of chlorophyll.

2.4.2 Effect of K on the greening process

In order to investigate whether or not cytokinins were involved in the greening process, leaf segments were incubated in a solution of K at 5 mg l^{-1} during, or prior to exposure to the light. 500 mg of leaf tissue, representing the amount of 12 replicate plant samples, was extracted for chlorophyll at time indicated. the data in

Figure 1.19

a



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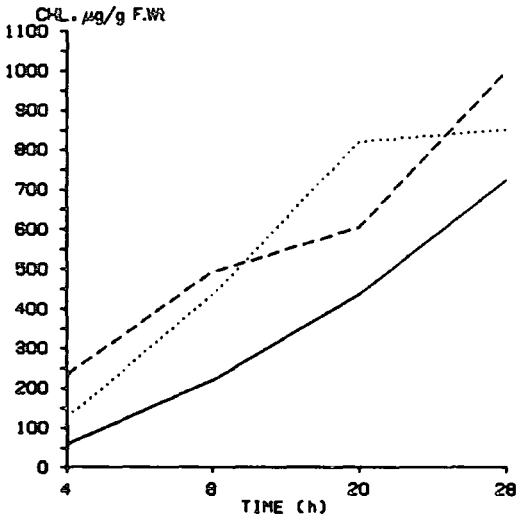

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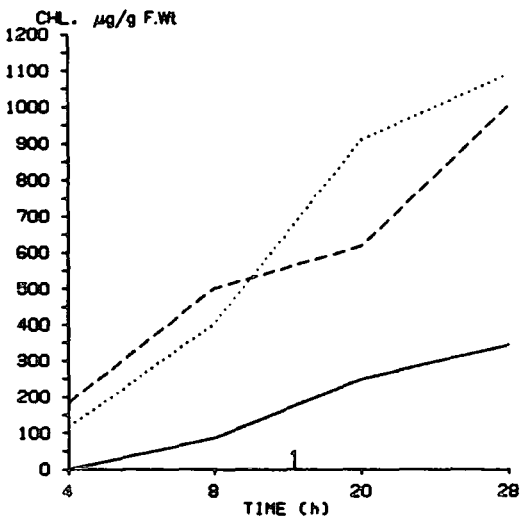
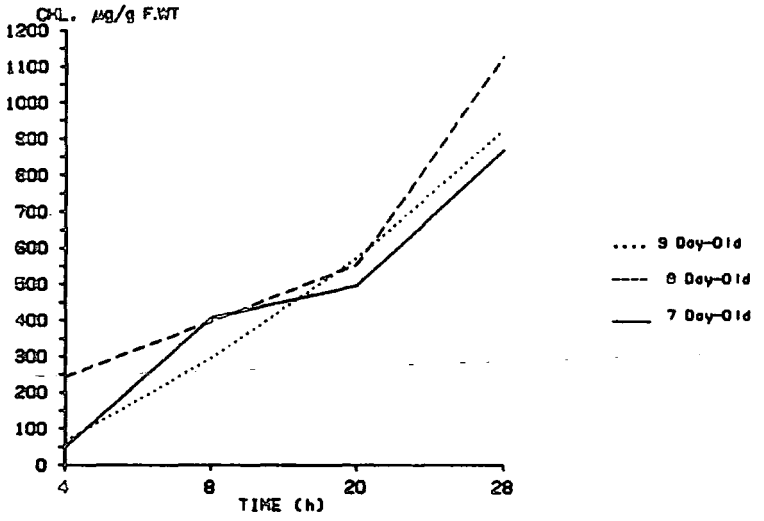


Figure 1.20

a



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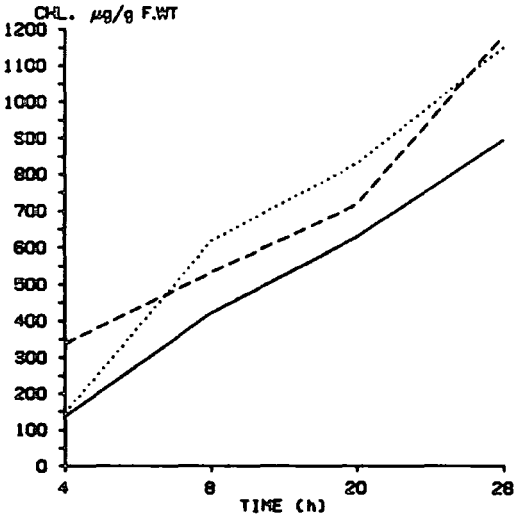

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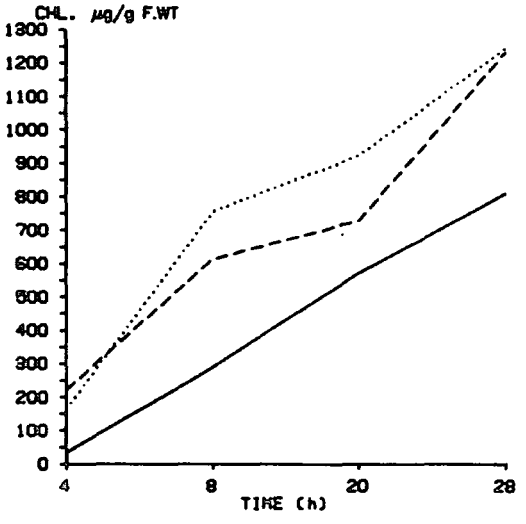
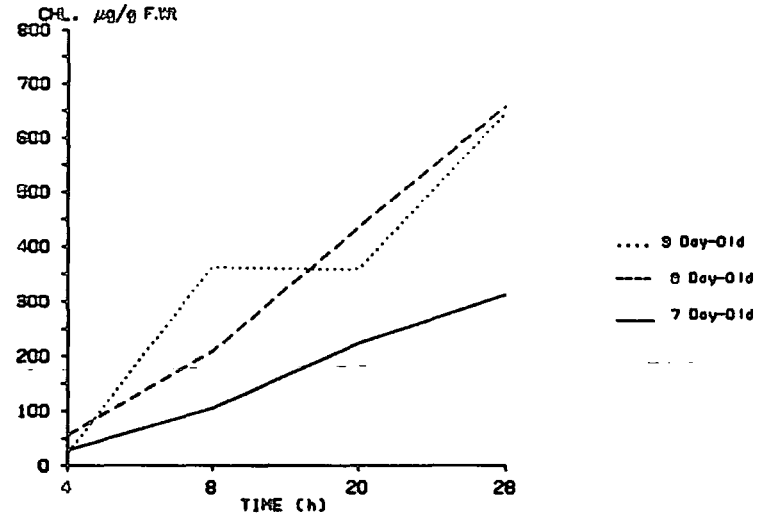


Figure 1.21

a



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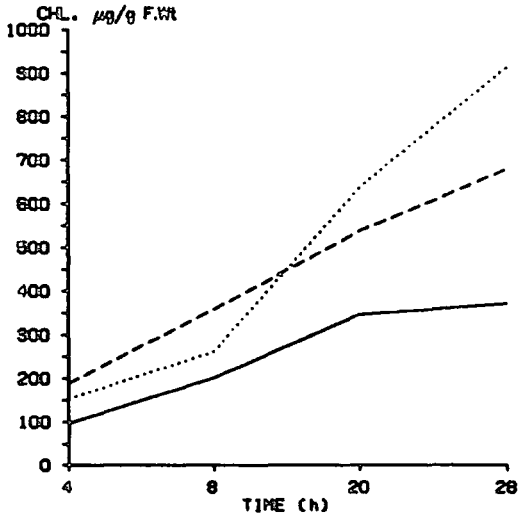

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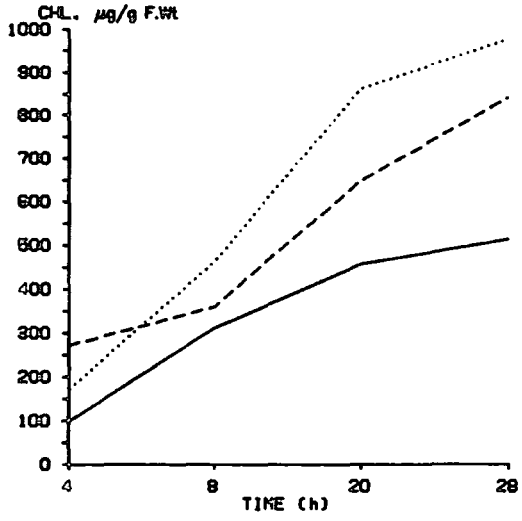
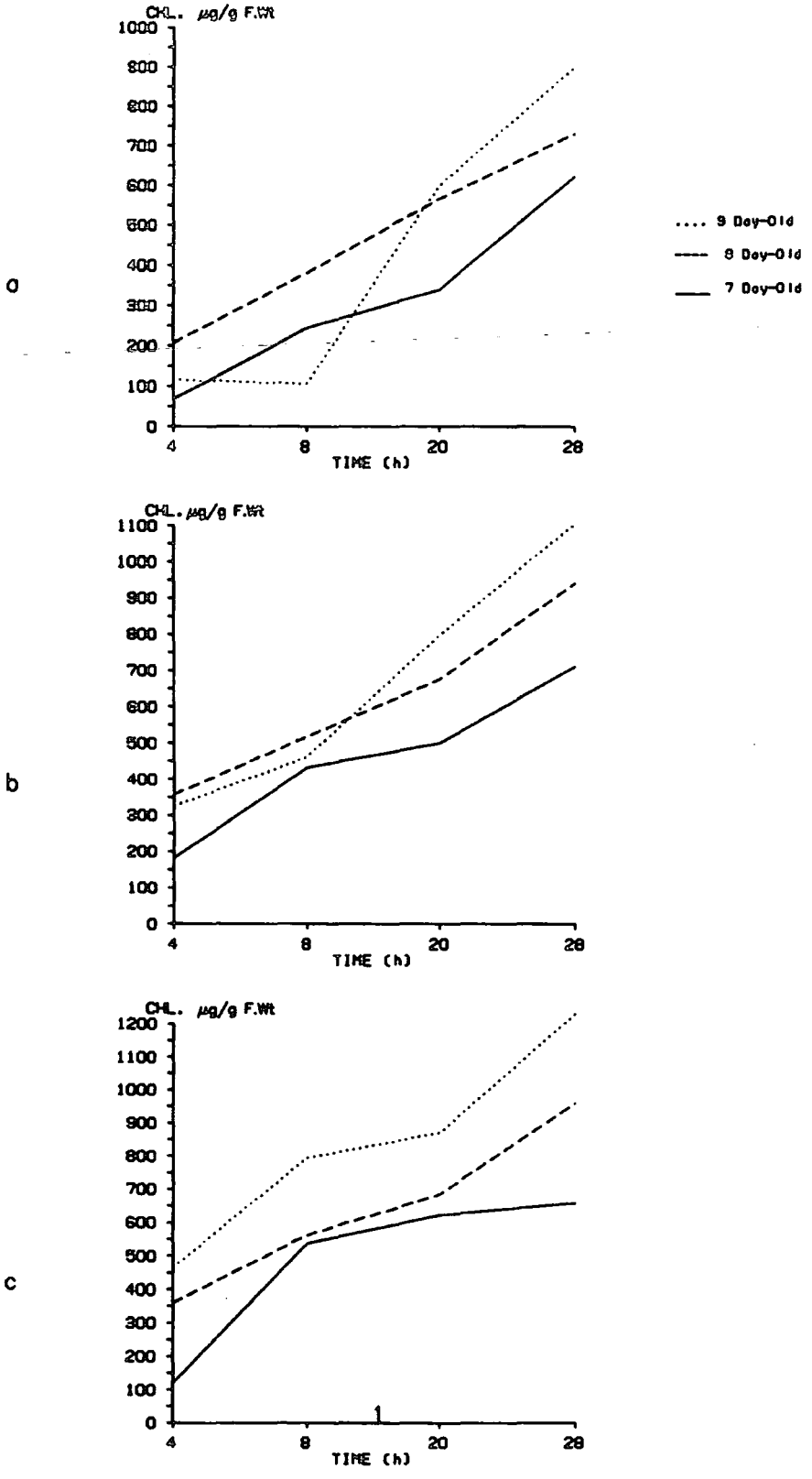


Figure 1.22


a
CHL. µg/g F.Wt
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4
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28
TIME (h)
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---- 8 Day-Old
—— 7 Day-Old
b
CHL. µg/g F.Wt
1100
1000
900
800
700
600
500
400
300
200
100
0
4
8
20
28
TIME (h)
c
CHL. µg/g F.Wt
1200
1100
1000
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700
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TIME (h)

Fig. 1.19 and 1.20 show that the differences in the pattern of greening in the tip, middle and base segments were maintained over 28 h and showed a similar pattern to that recorded above. K treated leaves appeared to have increased levels of chlorophyll in all segments but the pattern of differential greening was maintained (tip least and base most). It was noted that K appeared to have more effect on the greening of segments derived from young tissue (7 day-old) than from older tissue (9 day-old). This effect was noted for tip, middle and basal segments. The data in Fig. 1.21 and 1.22 show that K had a slight effect on the greening process of samples pre-incubated with growth regulator for 24 h prior to exposure to the light. However, pre-incubation with K did not affect chlorophyll levels.

2.4.3 Effect of water stress on the greening process

The effect of water stress on the greening process and any interaction with the age of tissue was investigated using whole seedlings, excised leaves and leaf segments. In this series of experiments, the chlorophyll content was calculated on a dry weight basis for the first leaf. This takes into account any changes in the fresh weight caused by the effect of water stress condition imposed by the PEG treatment. The data in Table 1.3 indicate that when 6 day-old wheat seedlings were incubated in the light with their roots in water for 48 h and 72 h, greening of the whole leaf occurred. More chlorophyll accumulated after 72 h incubation than after 48 h. The plants which were grown in the lower concentration of PEG showed a slight decrease in chlorophyll levels. However, at high concentrations of PEG, seedlings showed a marked reduction in the levels of total chlorophyll accumulated after 72 h. However, there was a slight increase in the levels of chlorophyll with this treatment applied to 10 day-old seedlings for 48 h. These seedlings exhibited a lower capacity to green than the 6 day-old seedlings when incubated in water, the capacity to green appearing to

Table 1.3

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	4.0	4.5	4.4	5.3	4.1	1.6
	72	6.3	5.9	4.2	3.0	4.5	2.5
10	48	1.6	1.8	2.0	1.6	1.6	1.5
	72	1.8	1.6	1.5	0.97	1.3	0.74

decrease with increasing age of the tissue. No marked difference was seen between the amount of chlorophyll accumulated in segments of 10 day-old seedlings incubated on water for 48 h and 72 h. Low osmotic strength PEG also increased slightly the level of chlorophyll accumulation in 10 day-old seedlings.

However, high osmotic strength of PEG (-10 bar) had an effect on the seedlings, over the same period of time, reducing the levels of chlorophyll. Furthermore, after 72 h incubation, wheat seedlings contained lower levels of chlorophyll in both osmotic concentrations of PEG, the chlorophyll levels being lowest at high osmotic concentration of PEG. These results are consistent with the results in Fig. 1.17. It is possible that water stress has a secondary effect on the leaves when the seedlings are treated via the roots. The effect of PEG on excised leaves was studied in order to establish whether or not stress effects on the leaves were secondary as the effect appeared to be on growth.

The data in Table 1.4 show that when 6 day-old, excised leaves were incubated in water for 48 h and 72 h, chlorophyll accumulated; however, the level at 72 h was slightly less than that at 48 h. Therefore, the presence of roots appears to influence chlorophyll accumulation. Under water stress condition, the low concentration of PEG had no effect on the chlorophyll level of 6 day-old tissue. However, it was noted that a greater reduction in chlorophyll level occurred at the highest PEG concentration. In contrast, 10 day-old, excised leaves incubated for the same period of time, showed a reduction in total chlorophyll levels using water and also with both PEG concentrations (Table 1.4).

The effect of water stress on leaf segments was investigated in order to establish whether or not the lack of effect of PEG on excised leaves could be due to tissue interaction. The first leaves of 10 day-old etiolated seedlings were cut into

Table 1.4

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	2.7	2.2	2.4	2.6	2.6	2.3
	72	2.1	2.8	2.4	1.7	2.3	2.2
10	48	1.4	2.2	2.1	1.7	1.7	1.4
	72	1.2	1.2	1.3	1.2	1.6	1.4

Table 1.5

Leaf segments	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
Tip	48	0.93	0.88	0.71	0.58	0.76	0.66
	72	1.2	1.2	0.91	0.42	1.0	1.1
Middle	48	1.4	1.8	1.8	1.5	2.0	1.8
	10	1.6	1.6	1.8	1.3	2.0	2.1
Base	48	2.7	2.4	2.2	1.8	2.4	2.1
	72	2.8	4.3	3.2	2.1	2.7	2.6

three segments of equal size prior to transfer to the light. The data in Table 1.5 show that the sequence of greening from tip to base was as mentioned before. Total chlorophyll was low in the leaf tip followed by middle segments. The highest levels of chlorophyll accumulated in the basal segments during 48 h or 72 h incubation in water. In contrast, When segments were treated with either concentrations of PEG, greening followed the same sequence as for water treated tissue, but effects on the segments were variable. However, it appeared that PEG caused a general reduction in chlorophyll for most leaf segments during the period of incubation. The greatest reduction, however, in chlorophyll levels occurred in the tip segments. It appears that the effect of PEG on greening was most marked and consistent when whole seedlings were incubated. This indicates a role of the root system. Therefore, no effect of water stress was observed on leaf area or excised leaves.

2.4.4 Effect of K and PEG on the greening process

The effect of the growth regulator and water stress on chlorophyll accumulation was investigated. The data in Table 1.3 demonstrate that there was no difference between the effect of a combination of K and PEG, or PEG alone, on chlorophyll accumulation at low concentration of PEG. K did not appear to overcome the effect of PEG. However, at a high PEG concentration, K was found to enhance the effect of PEG on the inhibition of chlorophyll accumulation. A similar reduction in the chlorophyll levels was found using 10 day-old seedlings (Table 1.3). This suggests that the presence of roots influenced the effect of PEG and K.

This was further investigated using excised leaves. It was observed (at both PEG concentrations) that K and PEG had no effect on chlorophyll levels when they were applied directly to the leaves (Table 1.4) either with 6 or 10 day-old tissue.

The results using leaf segments did not show a consistent pattern (Table 1.5). The sequence of greening from tip to base, however, was maintained in all treatments as was seen previously.

2.4.5 Effect of water stress on unrolling and greening of wheat leaf segments

The pattern of greening for whole seedlings could have been due to variations in the unrolling of the leaf tissue and the effect of water stress on this process. If a part of the leaf remained tightly rolled then the filtration of the light could influence the greening process. This possibility, when the tightly rolled dark-grown leaves may not green to the same extent when allowed to fully unroll upon transfer to the light, was investigated further.

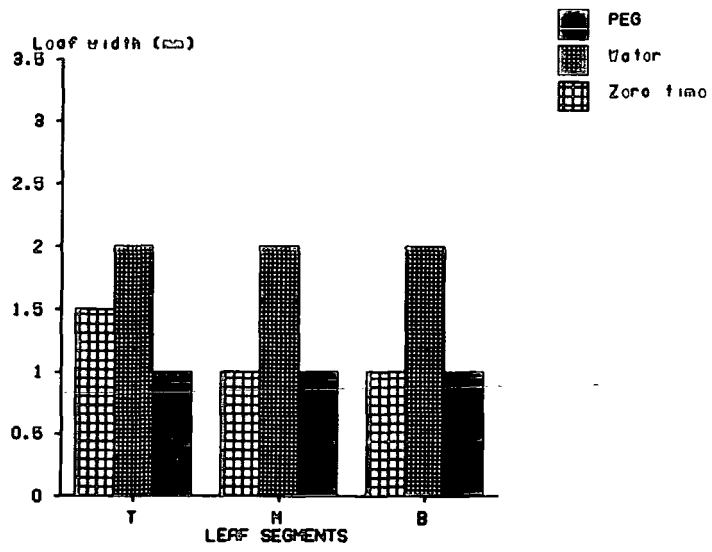
Wheat seedlings were incubated in the dark for 10 days. First leaves were excised and divided into three equally-sized segments (tip, middle and base). Average width of rolled segments was measured prior to their incubation with or without PEG at a high concentration, for 24 h, 48 h, and 72 h in the light.

In another series of incubations segments were kept for 24 h in water or PEG (-10 bar). After 24 h of incubation, the segments from the water were then transferred to PEG solution (-10 bar). Whilst those originally incubated in PEG were transferred to water. Both sets and controls were incubated for a further 48 h and 72 h in the light. The initial incubation for 24 h was to allow unrolling to occur for all treatments, particularly in water. Leaf width was measured at the end of the incubation and chlorophyll was estimated after each period of incubation for each of the segments.

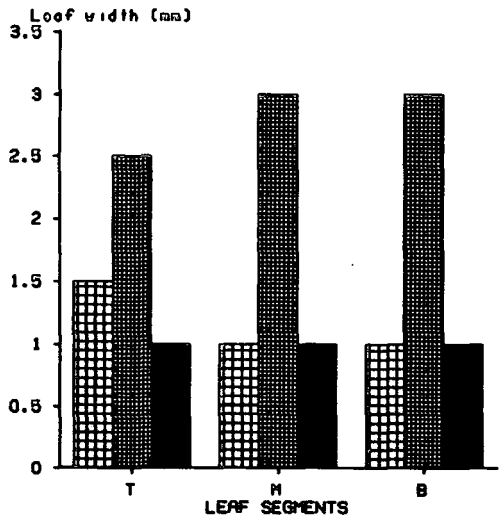
The data in Fig. 1.23a show that leaf width was higher in all segments after incubation in water for 24 h in the light. However, the width of the tip segments was higher because it was slightly unrolled in the dark. In contrast, leaf segments were rolled after 24 h of incubation in PEG in the light and leaf tip was more tightly rolled than at zero time (Fig. 1.23a). The greening pattern after 24 h in the light followed

Figure 1.23

a



b



c

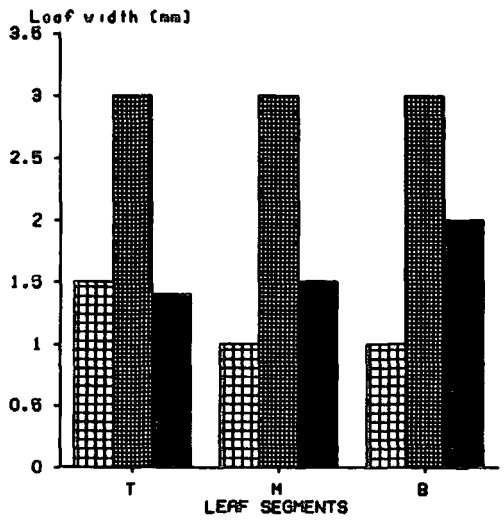
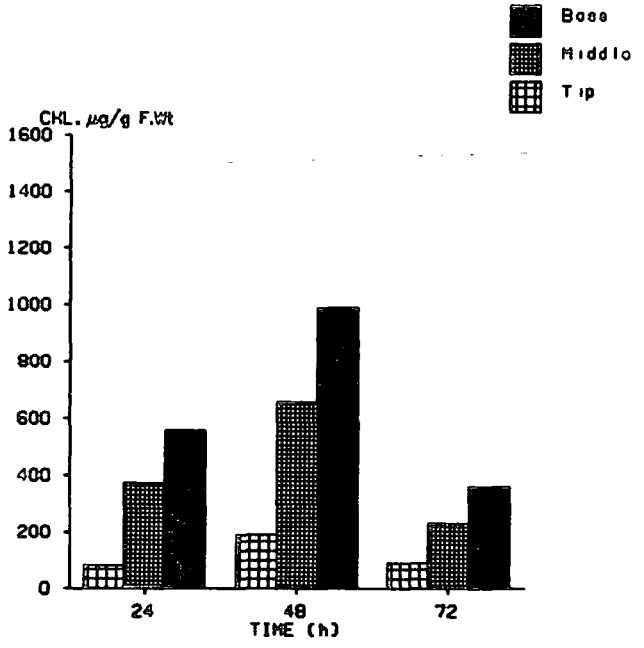
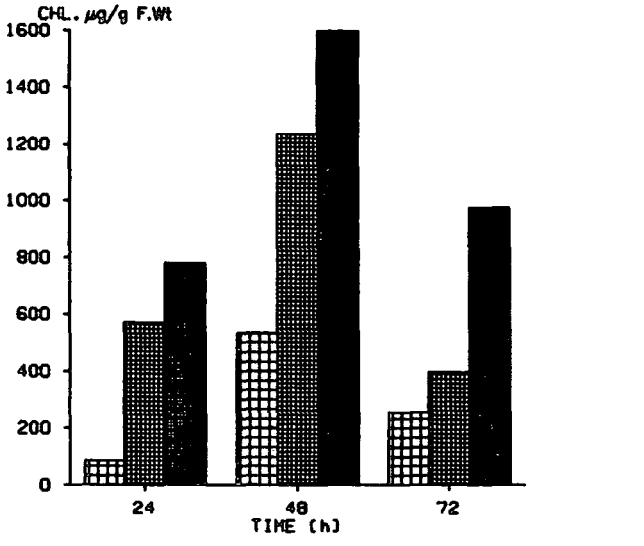


Figure 1.24

a



b



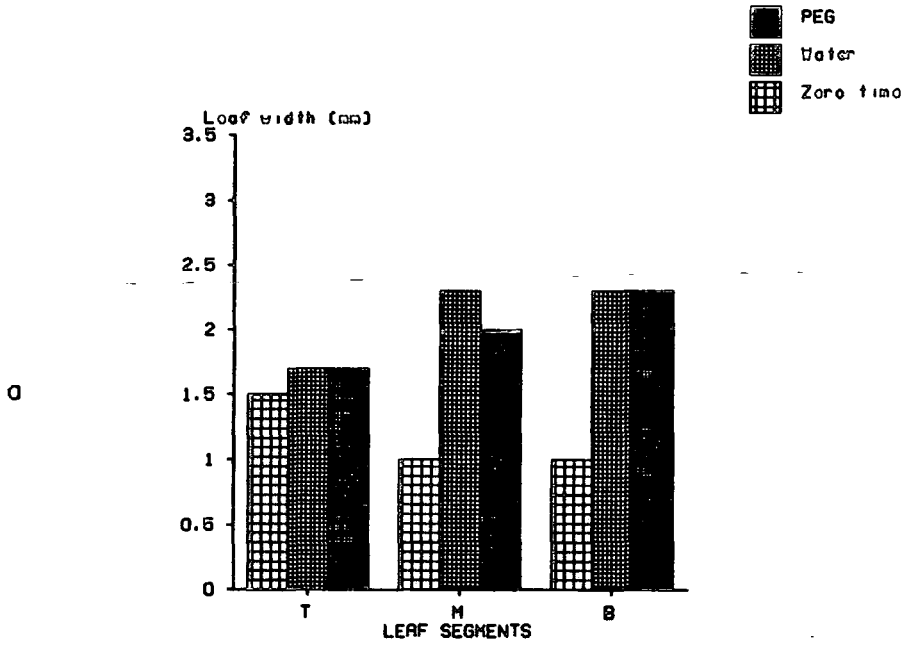
the same pattern from tip to the basal segments as mentioned before (Fig. 1.24a and b). After 48 h of incubation in water in the light, the segments were fully unrolled. PEG prevented leaf unrolling in all treatments, and their width was the same as at zero time (Fig. 1.23b). The greening after this period (48 h) of incubation was higher when segments were incubated in water than for 24 h. However, greening was lower in segments treated with PEG compared to the controls during sampling period (Fig. 1.24a and b). Although PEG prevented leaf segments from unrolling, the pattern of greening was maintained.

Unrolling occurred to a greater extent in all segments incubated in water for 72 h. PEG caused greater unrolling in basal segments than the other segments. However, it was less than with water (Fig. 1.23c). The greening was higher with water than with PEG, again as with the pattern seen previously.

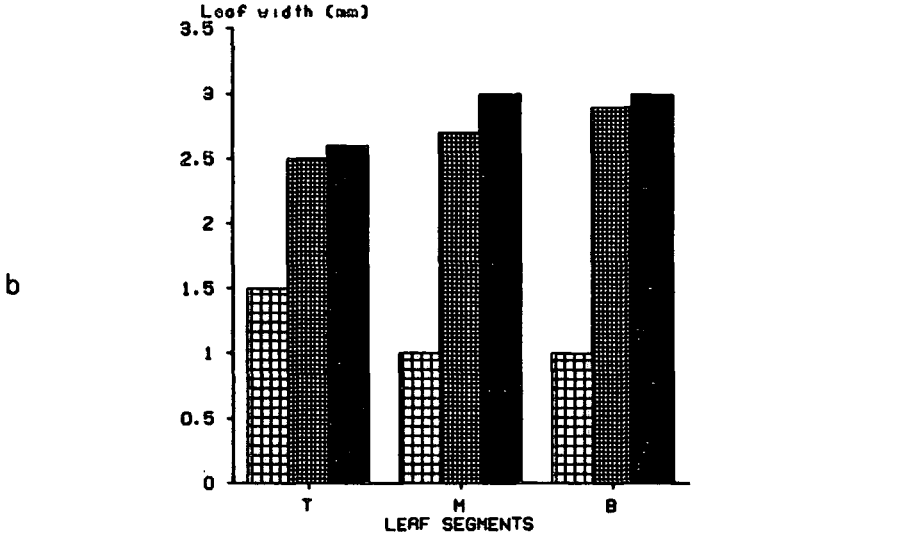
It was noted that the segments which were incubated in water then transferred to PEG for 48 h and 72 h, showed reduced unrolling of the tissue (Fig. 1.25a). When segments were transferred from PEG to water, for the same period of time, the segments showed a greater unrolling (Fig 1.25b) compared to those segments transferred from water to PEG. This indicated that the leaf segments were not totally rolled under water stress. At 72 h the same pattern of greening was found from tip to basal segments with those transferred to water than those with PEG solutions Fig. 1.25a and b.

These results indicate that PEG does not abolish the ability of the segments to green by an effect on unrolling of leaves. After 72 h of incubation, those segments transferred from water to PEG or from PEG to water showed the same pattern of greening from tip to the basal region (Fig. 1.26). The total levels of chlorophyll in all segments were less in those segments incubated in water then transferred to PEG,

Figure 1.25

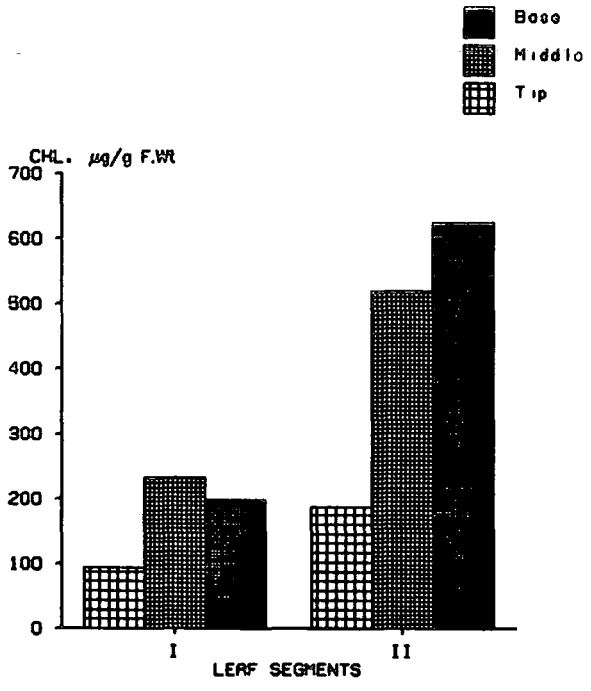


a



b

Figure 1.26


Base
Middle
Tip
CHL. µg/g F.WT
700
600
500
400
300
200
100
0
I
II
LEAF SEGMENTS

than for those segments transferred from PEG to water. However, all the segments which were incubated in PEG solution still have the ability to green.

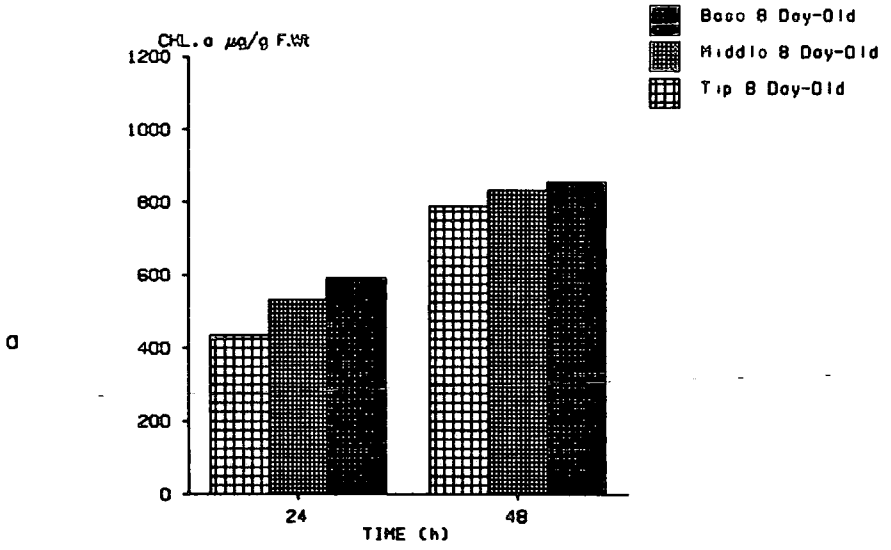
2.4.6 Chlorophyll *a* and Chlorphyll *b* during greening process

The rate at which the individual chlorophyll *a* and *b* components were produced was measured in one set of experiments. 8 day-old dark-grown leaves were excised and cut into three equally-sized segments (tip, middle and base). These segments were then incubated in the dark in water in Petri-dishes for 24 h prior to transfer to the light for 24 h and 48 h. At the end of this period the total chlorophyll was extracted and chlorophyll *a* and chlorophyll *b* contents were determined.

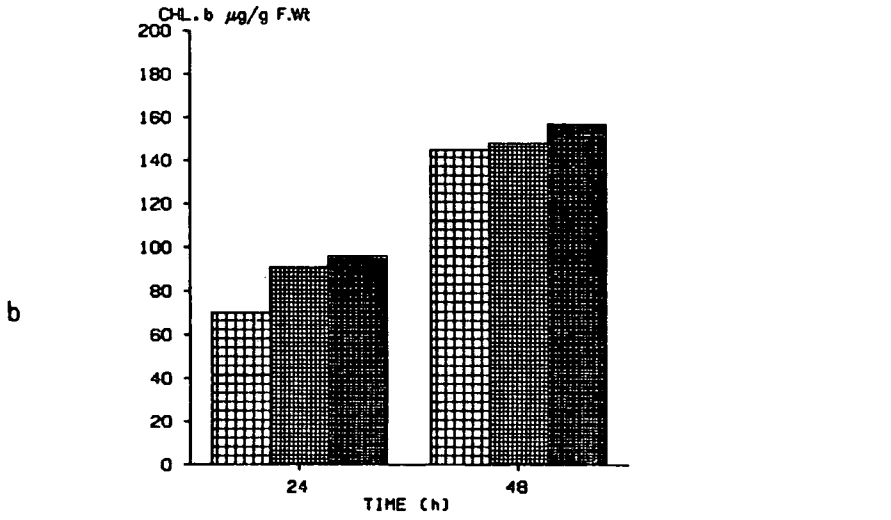
The results are shown in Fig. 1.27. It can be seen that for each segments there was a similar increase in chlorophyll *a* and chlorophyll *b* components from 24 h to 48 h of incubation. The rate of accumulation of these two components was more or less equal as can be seen from Fig. 1.27 a and b. Likewise the ratios between chlorophyll *a* and chlorphyll *b* were more or less the same as shown in Fig. 1.27 c.

The ratio of chlorophyll *a* to chlorophyll *b* was also investigated for 10 day-old intact wheat seedlings which were illuminated for 48 h and 72 h with their roots in water or -10 bar PEG. The results are shown in Fig. 1.28 where it can be seen that the water stress treatment did not alter the ratio of chlorophyll *a* and chlorophyll *b* at either 48 h or 72 h in relation to the control. There was a difference, however, in the absolute value of the ratio at these two times of incubation, it being higher at 72 h of incubation than at 48 h. These results are in agreement with those of Duysen and Freeman (1974) who found that water stress did not alter the ratio between the two chlorophyll components.

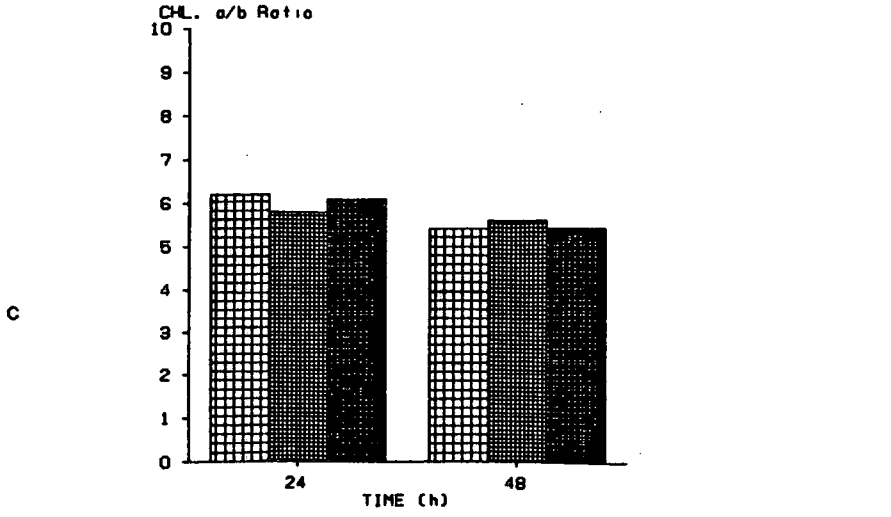
Figure 1.27



a

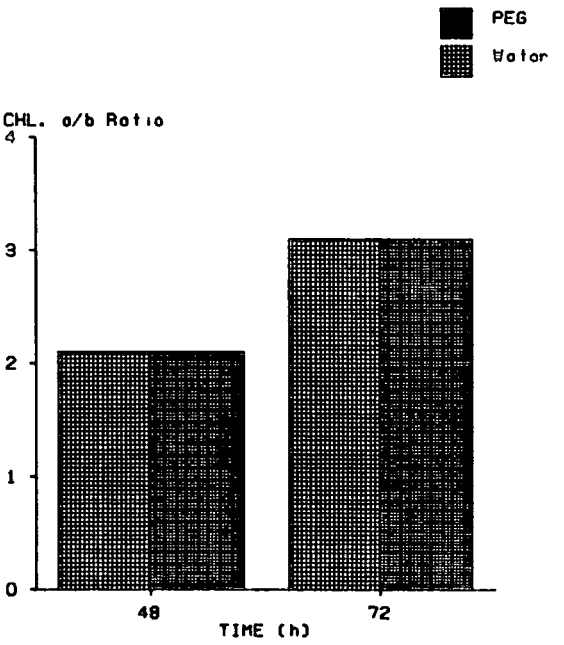


b



c

Figure 1.28



2.5 Discussion

One of the more striking effects of water stress on development of the first leaf of wheat seedlings was the inhibition of growth. The reduction in growth of the first leaf was more pronounced with both levels of osmotic stress in the older leaves compared with the younger ones. In contrast, it has been reported that the effect of water stress on growth tends to be especially pronounced in those tissues that are in a rapid stage of development (Williams and Shapter, 1955; Gates, 1968). Hsiao (1970) reported that plant growth was severely inhibited by water stress.

Many investigations have shown that cell division and cell enlargement are sensitive to water stress (Slatyer, 1967; Hsiao, 1970). Stransky and Wilson (1964) and Boyer (1968, 1970) found a decline in the rate of cell enlargement as water stress increased, with enlargement ceasing at moderate water stress. The knowledge that cell growth is generally more sensitive to water stress than stomatal opening (Hsiao, 1973) has a direct implication in the analysis of water stress effects on leaf growth. The effect of cell water stress will be apparent during the early stages of growth and development of the leaf whereas the effect on stomatal opening will be seen only after the stoma have developed in the leaf. Water stress at the early seedling stage, therefore, will influence greatly the establishment of the seedlings, in particular, the rate at which new leaves and hence photosynthetic capacity are produced. Mare and Palmer (1976) noted that the total number of leaves produced by the primary stem of sunflower was reduced when water stress was imposed over a period of 10 days.

In a review of water stress effects on wheat growth (Table 1.1 and 1.2), Hsiao (1973) concluded that leaf growth was very sensitive to water stress. It has been reported that older plants may stop growing in PEG solution because of the natural breakdown of the permeability of barriers with increased age (Macklon and

Weatherley, 1965). They also regarded root damage as a very important factor.

In this study, the reduction in leaf growth occurred in both light and dark incubations of 6 and 10 day-old wheat seedlings. 10 day-old seedlings. Christ (1978 a,b) has reported slow growth of wheat leaves in the dark. This may be due to a depletion of reserves in leaves grown at low photon flux densities. Davies and Van Volkenburgh (1983) reported that plant growth with a restricted water supply showed a reversal of the leaf growth pattern exhibited by leaves which were well-supplied with water. There was higher growth rates during the dark period than the light period. These observations suggested that the enhanced growth rates shown by soybean (Bunce et al., 1977) and sunflower leaves (Radin and Boyer, 1982) when the light was switched off may have resulted from the limitation of daytime growth by water stress. Radin (1983) has reported that nitrogen deficiency can greatly reduce the daytime rate of leaf expansion. Nitrogen deficiency apparently lowers the hydraulic conductivity of the roots leading to large daytime water stress. Delane et al. (1982) suggested that osmotic adjustment of cells during water stress conditions probably results in reduced growth rate of barley leaves. The effect could be that diversion of resoures for osmotic adjustment results in loss of these components being available for the leaf growth process. It is known from previous studies (Gates, 1955) that when young tomato plants were subjected to water stress, the growth of the plants was reduced. Leaves of different ages had different sensitivities to such stress. Young leaves suffered the greatest absolute growth reduction, but recovered most rapidly. Older leaves did not suffer such a great reduction in growth, but recovered from water stress more slowly (Gates, 1955).

It has been shown in the present studies that in intact wheat seedlings, K reduced leaf growth in both young and old plants. The reduction in leaf growth was

more noticeable in the light incubation treatment than in the dark treatment. It has been reported that cytokinins inhibit root growth (Miller, 1961) and may have partially inhibited radicle growth. Jones and Kaufman (1971) reported that K slightly promoted elongation of roots only in the absence of exogenously supplied substrate. In several other plant systems, such as sunflower hypocotyls (De Ropp, 1956) and pea stem segments (Brian and Hemming, 1957), exogenous K, when supplied with gibberellin in physiological concentrations, has the effect of suppressing gibberellin promoted longitudinal growth. The results here show that K in combination with PEG solutions, had no effect on the growth of the first leaf of wheat seedlings. The growth was inhibited with PEG, alone, as mentioned before.

The relationship between the shoot and the root is complex and homeostatic. When a change in the environment occurs, the rate of growth of one sub-system changes relative to that of the other so that the functional equilibrium is maintained (Richards, 1980; Troughton, 1980). Troughton (1980) suggested that there is maintenance of a constant carbon-nitrogen ratio so that if nitrogen supply from soil is increased, leaf expansion is promoted and carbon fixation is increased. Conversely, if assimilate supply is increased, root growth is promoted leading to enhanced uptake of nitrogen. Growth readjustments of this kind are relatively slow, but some experimental treatments, such as root cooling or removal, lead to a reduction in leaf growth which is detectable within hours (Davies and Van Volkenburgh, 1983; Carmi and Van Staden, 1983). It is often found that treatments applied to roots affect their capacity for water uptake; therefore, water stress at the roots has been invoked as being responsible for effects on subsequent growth in these tissues and also in other tissues of the plant.

The fresh and the dry weights for the leaves and the roots of 6 day-old

wheat seedlings incubated in the light and dark for 48 h and 72 h were compared. Comparison was made between the fresh weight for the shoot and the root system. The shoot decreased in growth to a greater extent than the root systems in the dark after 48 h of incubation, but it was higher in the light after 72 h. The higher concentration of PEG (-10 bar) was more effective in decreasing the fresh weight of both systems than the lower concentration (-5 bar). Similar responses occurred with the older seedlings (10 day-old) incubated in the dark and the light for 48 h and 72 h. The dry weight of shoot and root systems were slightly variable with PEG treatment depending on the tissue age and the period of incubation. There was slight increase in the dry weight for both systems of the young seedlings in the light than in the dark at 48 h of incubation. However, there was no effect of PEG on the root systems in both the light and the dark condition after 72 h of incubation. The dry weight of the leaves tended to decrease after PEG treatment in both the light and the dark condition in relation to the control after 72 h of incubation.

The dry weight of 10 day-old seedlings was higher with PEG for the roots, but it was the same for the leaves with PEG for the dark and the light incubation. It was reported by Mansfield et al. (1978) that the rapidity of the response suggested that either some kinds of stress signal, possibly hormonal, is rapidly generated and passed from the roots to the shoot resulting in reduced leaf growth, or that the treatment affects the generation or flux of a signal which is necessary for the continued expansion in control plants. The effects of reduced water supply on growing leaf tissue may not be simple. Water stress can affect cell division (Clough and Multhorpe, 1975) leaf metabolism (Hsiao, 1973; Lawlor and Leach, 1985), or may alter the biological parameters which govern cell enlargement, such as turgor, wall extensibility or wall yield stress (Tomos, 1985). It is possible that root treatments affect one or more of

these parameters directly and not through water stress.

The effect of K treatments on fresh and dry weights of the roots and leaves was variable. K had no effect on the fresh weight of roots of 6 day-old wheat seedlings incubated for 48 h and 72 h in the light and the dark. However, there was no effect on old tissue during 48 h of incubation, and there was no effect recorded after 72 h in the light and the dark. K did not overcome the effect of PEG with young or old tissue incubated in the light and the dark for the same period of time. The fresh weight of the leaves was reduced in both 6 and 10 day-old tissue incubated for 48 h and 72 h in the light and the dark except in the old leaves incubated for 48 h when the fresh weight was increased in relation to water. However, the effects of K with both levels of PEG were the same as for PEG alone treatment in the light and the dark with young and old tissue incubated for 48 h and 72 h. Similar responses were found with the dry weight of the leaves with K and a combination of K with both PEG concentrations. The dry weight of the leaves was reduced in the young tissue during incubation for 48 h and 72 h in both the light and the dark. However, there was no effect of K on the dry weight of the leaves. The effect of K on the older tissue was different. The dry weight of the leaves after 48 h was the same for water and PEG treatments, but after 72 h of incubation, there was a slight increase in the dry weight with K with both PEG concentrations. K itself gave no effect over PEG alone, where the dry weight of the leaves was reduced in the young tissue incubated in both the light and the dark for 48 h and 72 h. The dry weight was, however, slightly higher in the dark than in the light incubations. In the older tissue, K incubation with PEG solutions gave the same results as the control and PEG alone after 48 h of incubation in the light and the dark. After 72 h, the dry weight of leaves was slightly higher in the light than in the dark.

It has been shown by Richards, (1980) that cytokinin applied to the roots of apple seedlings resulted in markedly reduced growth. It was also reported that exposure of roots to K decreased the water uptake (Richards, 1980). Tal et al. (1970) and Collins and Kerrigan (1974) reported that cytokinins increased the resistance of the root to the absorption of water. The reduction in root growth depended on the amount of the root system treated with the hormone. In contrast, Srivastava (1967) reported that both fresh and dry weight of tissue growing on K media was greatly increased compared to plants growing without K.

In contrast, excised leaves of dark-grown leaves (6 and 10 day-old) had similar fresh or dry weights when they were treated with the same solutions for the same period of time as mentioned before. The treatments using excised leaves showed a slight increase in the fresh weight over the original weight. The dry weight was the same in all treatments. Similar responses were found with 10 day-old leaf segments when the same procedure was followed. These results suggest that the relation between root and shoot systems should be taken into account under water stress conditions. Therefore, there was a great effect of water stress, K treatments and PEG solution combined with K, directly on the root system which then affected the shoot system. It is well established that water stress and water-logging may lead to marked changes in endogenous levels of growth regulators in plant. These phenomena are documented for ABA (Wright and Hiron, 1969; Loveys and Kriedemann, 1973) and cytokinin (Itai and Vaadia, 1971). The interdependence between root and shoot function and growth is well documented (Boote, 1976). Wareing (1970) reported the involvement of root-produced hormones (which travel to the top of the plant) in hormonal control over shoot growth. Skene (1975) showed that cytokinins were the major hormone involved in this concept. Wittwer and Dedolph (1963) reported that cytokinins reduced top

dry weight and plant height, especially at a cytokinin concentration of about 2 mg l^{-1}. They also noted a different degree of inhibition exhibited by tops and roots resulting in decreased top:root ratios.

Leaves of higher plants grown in darkness lack chlorophyll and hence can not carry out photosynthesis. Upon illumination, the plastids of such leaves undergo a series of developmental changes, membrane components are reorganized, there is new synthesis of chlorophyll (Boardman et al., 1978; Bogorad, 1967; Rosinski and Rosen, 1972) and photosynthetic capacity develops (Bogorad, 1967; Anderson, 1975). In this study, it was found that the greening of primary leaves of 10 day-old dark-grown seedlings was not equal when they are transferred to the light for 72 h. The leaf tips were mostly yellow compared with middle and basal regions. However, the capability for greening under white light decreased from the apical sections to the basal ones along the whole leaf. This gradient remained unchanged during irradiation up to 72 h. This pattern followed the age sequence down the leaf where the leaf tips contained the oldest cells and the basal sections contained the youngest cells. This differential capability of greening may be due to a function of tissue interaction within the leaf. However, similar responses were found in excised segments with different ages. Chlorophyll accumulation decreased with leaf age and was higher in the basal segments. These results coincide with the findings from Virgin (1955) who showed that in old leaves the photoconvertible protochlorophyll is higher in basal sections of leaf than in the apical regions. Therefore, the capability of greening is higher in tissue of young leaves than of older ones. It has been reported that leaf age influenced the greening process in the dark grown barley, bean, and wheat leaves (Axelsson, 1977; Bradbeer et al. 1974). However, Bradbeer et al. (1974) reported that the rate of greening in Phaseolus vulgaris leaves increased gradually up to 14 days

after which the greening potential of the etiolated leaves declined. Accumulation of chlorophyll depends upon available food reserves. Detached bean leaves, which had lost their ability to green as a result of the depletion of substrates, regained the capacity to synthesize chlorophyll after incubation overnight with sucrose (Sisler and Klein, 1963). However, it has been reported that leaf age or stage of development is important, where older leaves may show a different degree of response than younger leaves (Raschke and Zeevaart, 1976).

Evidence for progressive senescence from tip to base after prolonged growth in darkness is the loss of greening capacity with increasing age, loss of fresh weight and loss of turgor (Obendorf and Huffaker, 1970). It has been reported that the effect of a growth regulator is associated with either a maintainence or enhancement of chlorophyll synthesis (Fletcher, 1969; Adedipe et al., 1971). When leaf segments of different ages were incubated with K solutions then transferred directly to the light or kept for 24 h in the dark, followed by exposure to the light for up to 28 h, chlorophyll levels increased in both treatments with all segments (tip, middle and base). However, the level of chlorophyll was higher in treated segments compared to control and the pattern of greening was as previously found from the leaf tip to the basal ones. The tip had the lowest levels and the base the highest levels of chlorophyll. However, K was more effective on young tissue rather than older tissue. Fletcher and McCullagh (1971) found that etiolated cucumber cotyledons, pre-treated with cytokinin and exposed to the light for 3 h had up to 45% more chlorophyll than the water control. The rapidity of the response indicated that cytokinin influenced chloroplast differentiation and chlorophyll biosynthesis. They also reported that the effect of cytokinins, in chlorophyll production, was a result of enzyme inductions including ALA synthetase. Ueda and Kuraishi (1977) reported that both transpiration and chlorophyll forma-

tion, in etiolated cotyledons of squash, were equally stimulated by K. These results are consistent with present observations that chlorophyll in the dark-grown wheat leaves was stimulated by K. However, Buchanan (1980) reported that the action of light in preventing pigment degradation of chloroplasts during senescence of attached and detached leaves is attributed to its action through phytochrome and/or through maintenance of proper hormonal balance. The role of light in controlling the biosynthesis of pigments in leaves cannot be ignored (Sironval, 1963). The decrease in the photochemical activities of chloroplasts caused by water stress during the greening of etiolated wheat leaf seedlings can be correlated with a decrease in the accumulation of chlorophyll. The decrease in chlorophyll accumulation in response to water stress has been reported for many species (Alberte et al., 1973; Nordin, 1976). The results indicated that the levels of chlorophyll accumulation during water stress conditions was reduced. The young tissue of wheat leaf seedlings showed a slight decrease in chlorophyll level with mild water stress after 48 h of incubation in the light. Severe water stress, using PEG solutions, causes the chlorophyll levels to markedly declined. However, old tissue exhibited the lowest level of chlorophyll accumulation with both PEG concentrations. The greening was lower in both young and old water stressed seedlings compared to the control seedlings. In contrast, Misra and Misra (1987) reported that the chlorophyll content, after a period of 48 h of illumination was approximately the same in stressed and unstressed leaves of 12 and 15 day-old seedlings. Also, the youngest one (9 day-old) showed a lower rate of chlorophyll synthesis compared to the control. They suggested that the youngest seedlings were relatively more prone to water stress than the older ones.

In different sets of experiments (Table 1.4 and 1.5), water stress was less effective on either excised leaves or leaf segments. The young tissue of excised leaves

was not affected by a low water stress of PEG (-5 bar). However, the effects of water stress was much higher with a higher water stress of PEG (-10 bar). Similar responses were observed with the old tissue of excised leaves. The effect of water stress on leaf segments (tip, middle and base) showed that there was no interactions between segments. The sequence of greening with water stress in the leaf segments from the tip to the base was observed as mentioned previously. The leaf segments responded differently to the water stress of both PEG concentrations. The segment most affected by water stress was the leaf tip followed by the other segments (middle and base). The pattern of greening was as in the control water, but it was more reduced compared with control samples. It has been reported that chlorophyll formation was reduced during water stress condition (Bengtson et al., 1978; Bourque and Nayler, 1971; Duyson and Freeman, 1974). Virgin (1965) showed that water stress had a greater effect on the synthesis of protochlorophyll than chlorophyll, such a result suggested that PEG-induced stress should have little effect on chlorophyll production until the protochlorophyll pool has been exhausted. Therefore, some chlorophyll could be produced even under water stress condition. In addition, it was reported that the chlorophyll content of leaves decreased rapidly during periods of water stress (Singh et al., 1972).

K treatments alone caused a very slight effect on the level of chlorophyll content in either young or old wheat seedlings during period of incubation. K and both PEG concentrations caused a reduction in the level of chlorophyll with both young and old seedlings, but the reduction was more pronounced with a combination of high PEG concentrations compared to the lower one. Furthermore, K treatments or K with both PEG concentrations had no major effect on chlorophyll levels in young and old excised tissues. The treatment of PEG and K had no effect on leaf segments

(tip, middle and base). The level of chlorophyll was variable in all segments with both K and K with PEG (low and high) concentrations. The pattern of greening down the leaf was observed in all treatments. Itai and Vaadia (1965) reported that the cytokinin concentration of root exudate from sunflower plants was markedly reduced after a 24 h period of water stress. Water stress may have caused leaf senescence by reducing the production of cytokinin in the roots and, consequently, reducing the cytokinin supply to the shoot. Extraction of cytokinins would be necessary to establish this, but this was not carried out in the present study. K treatments may have overcome the loss of the ability of ageing dark-grown leaves to produce chlorophyll upon exposure to light. Wolf (1977) reported that the rate of chlorophyll accumulation was influenced by many chemical agents including cations and plant growth regulators. Potassium and calcium had noticeable effects on chlorophyll accumulation (Knypl, 1969; Knypl and Rennert, 1970). The former promotes chlorophyll accumulation and the latter inhibits chlorophyll accumulation. Knypl and Rennert (1970) showed that calcium inhibited both growth and chlorophyll accumulation in excised cotyledons of cucumber during a long period of illumination and that both types of inhibition were completely reversed by potassium. Green and Muir (1978) reported that a combination of potassium and calcium promoted chlorophyll formation in 7 day-old cucumber cotyledons.

In dark-grown wheat seedlings, the first leaf remained tightly rolled even when it emerged from the coleoptiles. However, upon illumination this leaf unrolled rapidly (only the tip region started to unroll in darkness as the leaves increased in age). Virgin (1962) demonstrated that red light was effective in promoting leaf unrolling. Leaf unrolling can also occur by phytochrome-controlled changes in the endogenous levels of gibberellic acid (Beevers et al., 1970; Poulson and Beevers, 1970; Cooke and Saunders, 1975 a,b). ABA prevented light stimulated unrolling (Poulson

and Beevers, 1970). When the leaf segments of dark-grown wheat were floated on water in the light for 24 h there was a marked increase in their width, and chlorophyll content. This increase in width is similar to that reported by Beever et al. (1970), and Pearson and Wareing (1970) when the leaf segments were illuminated for 10 min with red light followed by darkness. More recently Viner et al. (1988) reported that unrolling etiolated barley leaves had a calcium requirement.

Incubation of the leaf segments in the light for 24 h in PEG (-10 bar) solutions resulted in an inhibition of both unrolling and chlorophyll accumulation. However, transferring the leaf segments, to the water for 48 h and 72 h in the light, resulted in an increase in both leaf width and chlorophyll content. In contrast, transferring the leaf segments from water (after 24 h in the light) to PEG solutions in the light for the same period of time caused a reduction in both the leaf width and chlorophyll content. Despite, the inhibition of unrolling by PEG solution, the pattern of greening down the leaf was maintained either in water or PEG solution.

From the above discussion, it would appear that the unrolling of wheat leaf segments in water (in the light) is associated with an increase in the levels of chlorophyll. The effect of PEG appears to be both on the unrolling and greening here since, even when the leaves are pre-unrolled, PEG still reduces chlorophyll accumulation.

CHAPTER THREE

PROTEIN

3.1 Introduction

Leaf proteins are the major store of nitrogen. They act in a structural capacity and function as catalysts such as ribulose biphosphate carboxylase (RuBisCo). The proteins of plant cells are distributed between several cellular components and organized bodies. In leaves, most of the protein is located in chloroplasts (Stahmann, 1963; Huffaker, 1982). The remainder is distributed between the nuclei, mitochondria, microsomes, cytoplasmic protein and combined with cell wall material (Perei, 1959; Stahmann, 1963). Wildman and Bonner (1947) and Wildman et al. (1949) reported that the cell-free protoplasm from tobacco leaves consisted of three types of nitrogenous material. Firstly, the soluble low molecular weight nitrogenous components such as amino acids. Secondly, the particulate matter such as chloroplasts and thirdly, the soluble protein of the leaf cytoplasm. It has been found that the amount of soluble cytoplasm protein ranges from 23% to 50% of the total leaf protein (Wildman and Cohen, 1955; Boulter et al., 1972).

The leaf storage proteins are important in the maturation, reproduction and final seed yields of plants. Nitrogen can limit photosynthetic capacity and hence seed yield, as the plant matures. Final seed yields depend on the proteolysis of stored leaf nitrogen and its translocation to the seed (Dalling et al., 1975; Hageman and Lambert, 1981).

RuBisCo is an important factor in metabolism in plant leaves. It is responsible for the initial stage of photosynthesis and photorespiration. It is present in high concentrations in the leaf. RuBisCo constitutes from 40% to 80% of the total

soluble leaf protein of soyabean Glycine max and alfalfa Medicago sativa (Huffaker and Miller, 1978; Wittenbach, 1979; Friedrich and Huffaker, 1980), and from 10% to 30% of the total leaf protein in corn Zea mays (Huffaker and Muller, 1978; Wittenbach, 1979; Friedrich and Huffaker, 1980). The level of RuBisCo depends on the species and environmental conditions (Huffaker and Miller, 1978; Wittenback, 1979; Friedrich and Huffaker, 1980). It is estimated that the loss of RuBisCo is about 80% of the total loss of soluble protein in wheat seedlings grown in the dark (Wittenbach, 1978). It has been found that RuBisCo belongs to a group of enzymes which decrease rapidly in concentration at the senescence stage, whilst other enzymes decline more slowly (Batt and Woolhouse, 1975).

RuBisCo is made up of two different subunits:-

1 - a large subunit (mol.wt ca 48000-55000).

2 - a small subunit (mol.wt ca 1400).

The large subunit is synthesized on 70S polyribosomes in the chloroplast (Hartley et al., 1975; Alscher et al., 1976; Boulter et al., 1972) and the small subunit is synthesized on 80S polyribosomes in the cytoplasm (Chua and Schmidt, 1978; Gooding et al., 1973; Highfield and Ellis, 1978). The small subunit is transferred to chloroplasts as its precursor and combined with the large subunit into a native enzyme (Highfield and Ellis, 1978; Chua and Schmidt, 1978). Ellis and Hartley (1971) and Kleinkopf et al. (1970) reported that etiolated plants contained small amounts of RuBisCo which increase in the level in the presence of the light due to the onset of the development process. Brady and Steele-Scott (1977) found that the capacity for RuBisCo synthesis decreased with leaf age. Peterson et al., (1973) noted that the increase in the levels of RuBisCo remained stable until the leaf reached the

senescence stage then the RuBisCo disappeared rapidly with up to a 76% decline in barley Friedrich and Huffaker (1980)

Protein concentration has been reported to be influenced by internal factors such as tissue age and plant growth regulators (Strain et al., 1971). It has been reported that most leaves show a general decrease in their protein contents upon maturation (Pirie, 1955; Webster, 1959; Shah and Loomis, 1965). Similar results were reported by Mothes et al. (1958) using tobacco leaves and Samuels (1959) using sugar cane. Hendry and Stobart (1977) using etiolated barley leaves, reported that the levels of protein and amino acids decreased with plant age.

Plant growth regulators have a significant effect on protein concentration as mentioned before. Vaadia et al. (1962) reported that cytokinins prevent reduction in protein levels and retard ageing. Similar results were reported by Mothes (1964). Richmond and Lang (1957) and Osborne (1962) demonstrated that protein levels in detached green leaves of Xanthium pennsylvanicum were retained by cytokinin. Srivastata and Ware (1965) and Boer and Feierabend (1978) found that cytokinins caused an increase in the levels of ribosomes and polyribosomes in green tissues. It has been reported that cytokinins increase the ratio of chloroplast ribosomes to cytoplasmic ribosomes (Takegami, 1975; Boer and Feierabend, 1978).

It has been reported that protein metabolism is influenced by external factors such as light and water supply (Stewart and Durzan, 1963). Lyttleton (1962) reported that the levels of soluble proteins increased after 20 h when etiolated wheat leaves were illuminated. Graham et al. (1968,1971) reported that red light increased the activity of RuBisCo (91 fold) in pea seedlings after 5 days illumination. Similar results were reported by Chen et al. (1967) with etiolated corn leaves. The ratio of RuBisCo to ribulose bisphosphate carboxylase activity of barley plants remained constant after

24 h of the greening process in white light. It has been reported that light enhances the synthesis of the large subunit of this enzyme in fully greened fronds of Lemna minor (Blackwood and Leaver, 1977).

The inhibition of protein synthesis is an early response to water stress (Hsiao 1973). Bewley et al. (1983) reported that water stress elicits quantitative changes in protein synthesis in plants. Bidwell and Yeman (1956), working with carrot root explants, reported that amino acids arise by protein breakdown while they are re-utilized for protein synthesis. Protein synthesis and breakdown take place in separate compartments of the cell. Similar results were reported by Ryan and Walker-Simmons (1981). Barnett and Naylor (1966) found that soluble and total protein content declined in Bermuda grass under water stress. Similar results have been reported by Shiralipour and West (1984a) with maize seedlings. Similarly, Maranvill and Paulson (1972) reported that seedlings of corn showed a reduction in protein synthesis, instead of increased protease activity, causing leaf protein concentration to decrease during severe water stress, but significant decreases in proteins were not found during mild stress. Subbotina (1962), working with excised and attached leaves, reported that wilting led to an increase in the amount of soluble protein. Protein turnover can lead to losses of up to 40% to 60% of the leaf protein during water stress (Hanson and Hitz, 1982).

One of the major responses of plants to water stress is to synthesize nucleic acids (Kessler and Tishel, 1962). Thus it would appear that this links to the synthesis of proteins which are renewed during water stress for the resumption of cellular activity when the stress is relieved (Henckel, 1970).

Amino acids play an important role in plant nitrogen metabolism and exhibit a close relationship with protein metabolism (Tan and Habloran, 1982; Fukutaka and

Yamada, 1984). It has been reported that free proline accumulates in leaf tissue in response to water stress in intact plants (Chen et al., 1964; Barnett and Naylor,1966; Singh et al., 1973a; Waldren and Teare, 1974) and excised leaf tissue (Kemble and MacPherson, 1954; Palfi, 1968). The amount of proline that accumulates varies with the degree of stress and species (Voetberg and Stewart, 1984). Aspinal and Paleg (1981) reported that proline accumulation is a fundemental response of living organisms to water stress. However, it has been reported that proline accumulation does not occur in some higher plants, drought-tolerant bryophytes, some pteridophytes and lichens under water stress (Stewart and Larther, 1980). It has been concluded that proline accumulation cannot be regarded as a universal response of plants to water stress (Palfi and Juhasz, 1971).

It has been reported that proline accumulation may reflect a compensatory mechanism for better plant survival during a period of water stress. Such a conclusion is based on the theory that proline acts as an osmotic regulator (Aspinal and Paleg, 1981), a protector of enzyme denaturation (Paleg et al., 1980), a reservoir of nitrogen and CO_2 sources (Fukutaku and Yameda, 1984) and a stabilizer of the machinery for protein synthesis (Kardpal and Row, 1985). Ilahi and Dorffling (1982) reported that proline accumulation is mediated by ABA. Stewart and Voetberg (1985), using barley plants, reported that salt stress increased the levels of free proline in leaves without increases in leaves of ABA. Similar results were reported by Pomeroy and Siminovitch (1970). In agreement with the previous work, Parker (1962), using ivy leaves, reported that water soluble protein increased from summer to winter. Similar results have been reported by Gerloff et al. (1967) using alfalfa root and Morton (1967) using cabbage leaves.

This Chapter represents the results of investigations of the some of the

changes in soluble proteins during greening of etiolated wheat leaves. The effect of age and length of incubation in the dark prior to illumination was also determined. In addition, the effect of mild and severe water stress on protein accumulation was examined. Particular reference was paid to the relation of the differential greening response along the length of the leaf.

The attainment of a functional photosynthetic capacity within the leaf depends on the accumulation of functional proteins. However, given that protein components can change under water stress conditions it was important to establish the pattern of protein accumulation during greening in relation to water stress. In the broader context protein levels are important in the nutritional aspect of plants in general, particurly grasses, and any delay in achievement of the functional level would impair development and crop value.

In this study analysis only of the soluble protein was made which would exclude to a large extent any changes in structural components. However, this soluble fraction of protein appears to be more important in stress-related responses.

3.2 MATERIALS AND METHODS

3.2.1 Materials

3.2.1.1 Plant Material as described in General Materials and Methods.

3.2.2 Methods

3.2.2.1 Protein extraction

Leaf tissue (500 mg) from either 6 or 10 day-old plants was ground to a fine powder in the presence of liquid nitrogen, using a pestle and mortar. The leaf sample was then treated as follows:

a. Leaf sample was mixed with 1.0 ml extraction buffer (100 mM trizma base, 53 mM sodium acetate, 8.8 mM magnesium acetate, pH 7.2). The homogenate was then poured into a centrifuge tube.

b. A further 1.0 ml of extraction buffer was added to the pestle and mortar and the washings were combined with the homogenate in (a).

c. The homogenate was centrifuged at 2000 g for 10 min at room temperature (21°C) in a bench top centrifuge

d. The supernatant from (c) was decanted into a centrifuge tube containing 1.0 ml 10% (w/v) aq. trichloroacetic acid (TCA) and incubated in the dark at $4^{o}C$ for 30 min.

e. The solution from (d) was centrifuged, the supernatant discarded and 1.0 ml M NaOH added to the pellet.

f. The solution from (e) was incubated for 30 min in a water bath at 30°C and protein was then determined by the method of Lowry et al. (1951) using the

following reagents.

A. 2 g sodium potassium tartrate, 100 g anhydrous Na_2CO_3 in 500 ml M NaOH, distilled water to 1 L.

B. 2 g sodium, potassium tartrate, 1 g anhydrous $CuSO_4$ in 90 ml distilled water and 10 ml M NaOH.

C. Folin Ciocalteu's phenol reageant as 1 volume reagent : 15 volumes distilled water.

3.2.2.1.1 The Procedure:

1. 0.05 ml samples of the protein solution were taken and made up to 1.0 ml with distilled water.

2. 0.9 ml reagent A added, the solution shaken and incubated for 10 min. in a water bath at 30°C.

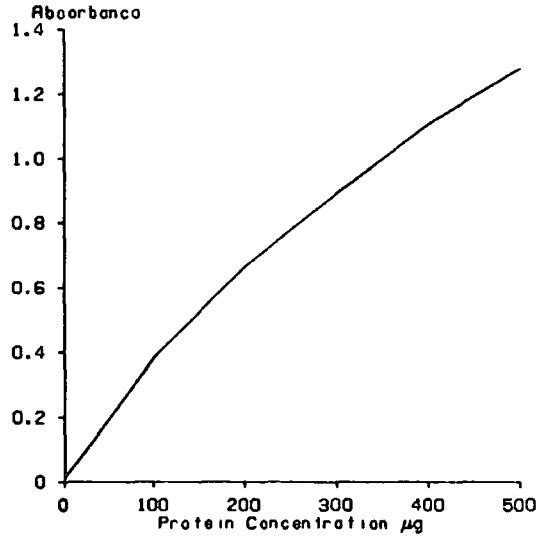
3. 0.1 ml reagent B was added, the solution shaken and incubated for at least 10 min at room temperature (21°C).

4. 3.0 ml of reagent C was added with immediate mixing using a Whirlimix. The solution was then incubated for 10 min. in a 30°C water bath.

Absorbance of the blue coloured solution was measured at 650 nm in the spectrophotometer (Ultrospec 4050, LKB Biochrom) against a blank consisting of 1.0 ml distilled water treated as the sample.

A calibration curve for protein concentration (Fig. 3.1) was prepared using BSA v made up in M NaOH. The samples of BSA v were treated along with the leaf tissue samples.

Figure 3.1


Absorbance
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
Protein Concentration µg

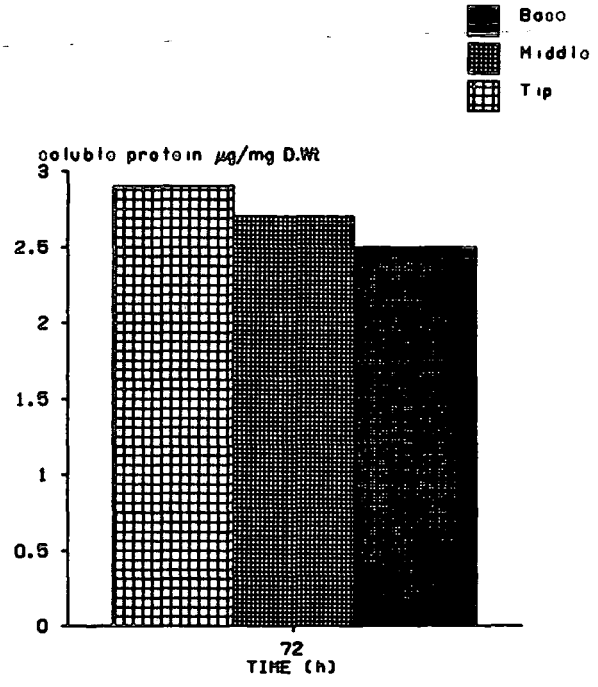
3.3 RESULTS

3.3.1 The effect of age of leaves on the soluble protein content during the greening process

Wheat seedlings were exposed to the light for 72 h following growth in the dark for 10 days. The first leaf was cut into three equally-sized segments (tip, middle and base). The different segments of the leaf were found to have variable levels of soluble protein (Fig. 3.2). The level of soluble protein was higher in the tip segments, followed by the middle segments with least being found in the basal segments. Therefore, the sequence of protein content of the leaf from the base to the tip was not consistent with the sequence of chlorophyll content as seen during the greening process. This pattern was not consistent with the expected pattern of the highest protein content in the basal segment. The high level of soluble protein in the tip segment could be the result of breakdown of insoluble protein. Changes in protein levels may be related to the age of the tissue in development terms and to changes in water status.

In order to investigate the above effect, experiments were carried out with wheat seedlings, excised leaves and leaf segments. Tissue of different ages was used with and without water stress treatment. The data in Table 3.1 and 3.2 represent the values for soluble protein from 6 day-old and 10 day-old wheat seedlings, which were incubated for 48 h and 72 h, and in the light and the dark. It can be seen that more soluble protein was present at 48 h than after 72 h, in water, in both light and dark. This was probably related to aging of the tissue where there could be a shift from structural insoluble proteins to the soluble forms by degradation.

Figure 3.2


Base
Middle
Tip
soluble protein µg/mg D.Wt
3
2.5
2
1.5
1
0.5
0
72
TIME (h)

3.3.2 The effect of water stress on the levels of soluble protein in wheat seedlings

The data in Table 3.1 and 3.2 show that when tissue was incubated in the light, increases in protein concentrations were observed in response to water stress. However, these changes appeared to be related to the age of the tissue. 6 day-old tissue showed a higher level of protein at the lower PEG concentration at 48 h in relation to the controls. However, the leaf tissue from seedlings under higher water stress showed protein contents which were essentially the same as the control. After 72 h of incubation, the level of protein was higher in both PEG treatments than in the controls even though the level for the tissue incubated on water from 48 h and 72 h declined. When 10 day-old tissue was incubated as above, there was essentially no change in the protein levels. In contrast, the level of soluble protein declined with the low PEG concentration after 72 h for tissue incubated in the dark for both 6 and 10 day-old leaf tissue (Table 3.2). However, in the 6 day-old tissue, the treatment with both PEG concentrations caused a higher level of soluble protein after 48 h. This was consistent with a response of the tissue to water stress where soluble protein can increase. After 72 h, the level of protein was lower indicating that the tissue had lost the ability to retain protein in response to stress. This effect is shown further by the 10 day-old tissue which was unable to accumulate protein in response to water stress treatment.

In order to investigate the effect of water stress further for the whole seedling, the levels of soluble protein were estimated in roots of intact seedlings. Roots of seedlings (6 and 10 days-old) which were grown either in light or dark for 48 h and 72 h, were used. The data in Table 3.3 and 3.4 show the protein levels in roots of seedlings, incubated with or without PEG in the light or the dark, respectively. There

Table 3.1

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	5.9	6.2	8.6	5.8	6.1	5.3
	72	3.6	5.0	4.6	5.8	5.0	7.9
10	48	2.4	2.6	2.5	2.3	2.2	2.1
	72	2.6	2.4	2.4	2.3	1.7	1.6

Table 3.2

Tissue age (day)	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	4.4	4.7	5.9	5.4	5.1	6.2
	72	3.4	2.7	2.3	2.8	2.6	2.1
10	48	3.8	3.0	2.8	2.5	1.8	2.1
	72	2.2	1.8	1.8	1.5	1.9	2.1

was a slight increase in the level of soluble protein in the young root tissue after 48 h of incubation in both PEG treatments. However, there was a decline in the level of soluble protein in the young tissue after 72 h. In the old tissue there was also a decline after 48 h and 72 h of incubation. Therefore, the roots exhibited the same pattern of protein levels as in the leaves for young tissue at 48 h of incubation, but the level of soluble protein declined with tissue age.

In order to investigate the potential role of the roots in supplying components for the leaf responses, experiments were designed involving treatments with K or a combination of K and PEG. The procedures and the periods of incubation were as in previous experiments (page 123).

K caused a slight increase in the levels of soluble protein in 6 day-old seedlings when they were incubated in the light for both 48 h and 72 h (Table 3.1). In contrast, there was very little effect of K on 10 day-old plants. The tissue may have lost its ability to respond to K treatment with ageing. However, when the tissue was treated in the dark with K, there was very little difference in levels of protein at 48 h for the 6 day-old tissue compared with the controls (Table 3.2). However, at 72 h the levels of protein in the tissue declined slightly with the K treatment but there was a general decline in all the treated tissues of this age.

Therefore, with old tissue the response to K, was reduced. However, with a combination of K and PEG, K showed no effect over PEG in the young tissue after 48 h of incubation. In contrast, with old tissue K enhanced the effect of PEG reducing the protein level in tissue incubated in both the light and the dark as shown in Table 3.1 and 3.2.

K gave a slight enhancement of the level of protein in young and old roots

Table 3.3

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	1.1	1.5	1.6	1.7	0.78	0.88
	72	1.1	1.1	0.51	0.45	0.66	0.66
10	48	0.56	0.61	0.37	0.43	0.83	0.65
	72	0.82	0.83	0.67	0.44	0.65	0.36

Table 3.4

Tissue age (day)	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	1.1	0.73	0.96	0.66	0.69	0.52
	72	0.95	0.55	0.72	0.63	0.83	0.71
10	48	0.50	0.67	0.56	0.43	0.50	0.50
	72	0.52	0.67	0.52	0.29	0.59	0.48

tissue at 48 h and 72 h (Table 3.3 and 3.4). However, K had no effect on the old root tissue after incubation for 72 h in the light (Table 3.3). In dark treatments, the levels of soluble protein decreased in the 6 day-old tissue from 48 h to 72 h of incubation, but K enhanced the level of soluble protein after 48 h and 72 h (table 3.4).

These responses of the leaves of whole seedlings, which had intact roots, was investigated further using excised leaves and leaf segments. The data in Table 3.5 show the response of the excised first leaves to treatment with PEG, K or a combination of both compounds. The levels of soluble protein found after incubation in the light was lower for both young and old excised leaf tissue than for whole seedlings. Treatment of the leaves with both concentrations of PEG reduced the levels of soluble protein in the young and old tissue after 48 h and 72 h in the light. However, the reduction was higher after treatment with the higher concentration of PEG. Similar results were found with the samples incubated in the dark for the same period of time (Table 3.6). It was noted that the leaf segments which were incubated in the light and dark for the same period of time with both PEG solutions gave similar patterns of reduced protein levels as for the whole leaves (Table 3.7 and 3.8), respectively. The data in Table 3.5 show the responses of excised leaves to K treatment. These differences between the K treatment and the water controls were only slight and highly variable. It appears that K treatment had very little effect, if any, on the levels of protein in the tissue incubated in the light (Fig. 3.5) or in the dark (Fig. 3.6). However, the levels of protein were generally much lower than those found in the leaves of intact seedlings (Fig. 3.1 and 3.2).

The effect of K on the level of soluble protein in leaf segments following incubation for 48 h or 72 h is shown in Fig 3.7 and Fig 3.8. The data in Table 3.7 show that K caused a slight increase in the levels of soluble protein for the leaf tip

Table 3.5

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	3.2	2.2	2.5	2.2	2.8	2.6
	72	3.0	3.7	2.9	1.6	3.1	3.1
10	48	3.2	3.6	2.9	2.7	2.8	2.9
	72	3.6	3.4	3.2	2.7	2.7	3.1

Table 3.6

Tissue age (day)	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	2.9	3.1	3.1	2.4	2.8	2.3
	72	3.3	3.6	3.2	2.4	3.2	3.0
10	48	4.1	4.3	3.6	2.5	3.5	3.3
	72	3.0	3.3	2.2	2.2	2.5	2.4

Table 3.7

Leaf segments	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
Tip	48	3.0	3.2	2.6	2.7	2.6	2.6
	72	3.4	3.9	2.9	2.7	2.8	2.8
Middle	48	3.6	3.4	3.0	2.7	3.2	2.4
	10	3.3	3.7	2.7	2.4	2.6	2.8
Base	48	3.2	2.9	2.8	2.6	2.9	2.1
	72	3.5	4.3	2.7	2.6	3.4	2.9

Table 3.8

Leaf segments	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
Tip	48	2.9	3.4	2.3	2.1	2.3	2.2
	72	2.0	2.9	2.1	1.7	2.2	2.1
Middle	48	4.1	4.2	3.3	2.5	3.3	3.1
	10	2.7	2.9	2.1	1.9	2.2	2.5
Base	48	3.3	2.9	2.3	2.3	2.8	2.2
	72	2.5	3.7	2.1	1.6	2.3	1.7

after 48 h and 72 h in the light. However, soluble protein in the middle segments decreased after 48 h, but slightly increased after 72 h. Similar responses were found with basal segments. The data in Table 3.8 show that the level of soluble protein in tissue treated with K and incubated in the dark slightly increased in the tip and middle segments after 48 h and 72 h of incubation. However, soluble protein levels in the basal segments slightly decreased after 48 h and increased after 72 h, however, this effect was marginal and probably not significant. The data in Table 3.5 show the effect of K, with a combination of PEG, on the level of soluble protein of excised leaves. A combination of K and PEG solutions caused slight increases in the level of soluble protein in the light compared to the K effect on young tissue after 48 h of incubation in the light. The levels of soluble protein decreased in young tissue after 72 h. However, the level of soluble protein decreased in both combinations after 48 h and 72 h. The data in Table 3.6 show a similar pattern for soluble protein levels for both young and old tissue incubated in the dark.

Leaf segments eventally showed similar patterns of protein levels as excised leaves when incubated with K in combination with PEG at both low and high concentrations. K and PEG treatments caused a reduction in the levels of soluble protein in all segments incubated in the light or dark as seen in Table 3.7 and 3.8, respectively. However, the response to PEG appeared not to be modified by co-treatment with K. It would appear that the response of leaves is most marked when the seedlings are treated via the root systems.

3.4 Discussion

In addition to the normal enlargement and greening process following illumination, it was shown (Rhodes and Yemm, 1968) that there was a consistent increase in the protein content of the leaves. When dark-grown wheat seedlings were illuminated for 72 h protein levels in the segmented leaves were seem to be variable (Fig. 3.2). The apical regions exhibited the highest levels of protein. Thereafter, the middle and basal segments. This result indicated that the protein level increased from the base to the apex of the leaf. However, protein content varied quantitatively in different regions of the leaf, during greening. For example, there was a noticeable increase in the soluble protein in the apical region of the leaf compared to the basal region. This sequence of soluble protein content in the greening of dark-grown leaves is inconsistent with the sequence of chlorophyll accumulation, where the apical region had the lowest levels of chlorophyll and the basal region the highest levels of chlorophyll.

Proteins vary in their individual rates of degradation, depending on either their physical properties (Dice et al., 1973; Acton and Gupta, 1979; Cooke and Davies, 1980) or their location in the cell. Barratt and Woolhouse (1981) showed that different fractions from Phaseolus spp. had different in vivo rates of degradation.

It has been reported that water stress, in most plants, causes a reduction in growth (Cleland, 1967; Gates, 1968; Shiralipour and West, 1968), which may be associated with an alteration of protein metabolism (Benzioni et al., 1967; Dhindsa and Bewley, 1977). It has been shown (Dhindsa and Cleland, 1975) that water stress causes a change in the types of proteins produced by Avena spp. coleoptile cells and also a reduction in the rate of protein synthesis. Cooke et al. (1979a,b) found that when Lemna spp. fronds were placed under water stress, there was a reduction in growth and protein synthesis and an increase in protein degradation.

When PEG solutions were applied to the roots of 6-day old wheat seedlings, the soluble protein levels were increased with mild PEG (-5 bar). However, there was no increase with severe water stress in relation to the control after 48 h of incubation in the light. However, after 72 h of incubation the soluble protein level was higher with both PEG osmotic solutions. Old seedlings responded in a different way to water stress. They exhibited a greater reduction in soluble protein levels with different PEG concentrations after 48 h and 72 h, in the light. The incubation of wheat seedlings in the dark gave similar patterns of protein degradation. However, the reduction in levels of protein was higher than in those samples incubated in the dark. The soluble protein loss was increased in dark-incubated old leaves compared to those leaves incubated in the light. Such an effect may have been due to irreversible damage to the protein synthesizing complex (Bewley and Dhindsa, 1977). It has been shown (Shiralipour and West 1968) that under moderate water stress, protein synthesis was inhibited and under severe water stress degradation of protein occurred (such as maize shoots). Dhindsa and Clelend (1973) have shown that water stress caused a differential inhibition of the synthesis of some proteins.

The changes in the soluble protein which occurred in water-stressed roots of wheat seedlings under the condition described earlier was different from those in leaves. The level of soluble protein slightly increased in young tissue after 48 h of incubation in the light with both PEG solutions. However, after incubation of up to 72 h in the light, a decrease in level of soluble protein occurred especially with severe water stress. Similar responses were found in old leaves incubated in the light for the same period of time as young leaves. Obendorf and Huffaker (1970) reported that responses of RUDP carboxylase activity and soluble protein content to illumination were highest in leaves of 5 to 7 day-old barley plants and later declined. Furthermore,

when root of wheat seedlings was held in the dark under water stress condition, the response was rapid, and the soluble protein was reduced in the young and old tissue.

However, this study showed that water stress had no effect on either young or old excised leaves after 48 h and 72 h in the light and dark. The level of soluble protein remained the same in all treatments with small differences between the samples, which may be due to the stages of development and the time of incubation. Similar responses were found with leaf segments.

The effects of water stress on protein level has been reported by many workers (Hsiao, 1970; Morilla et al., 1973). Leaves subjected to water stress had an impaired capacity to make protein and showed a rapid loss of polysomes and an increase in monosomes. Responses to a continuing water stress included premature senescence involving a net loss of protein from the leaves (Tung and Brady, 1970). Todd and Basler (1965) found that there was a disappearance of protein in the chloroplast and mitochondria fractions with increasing water stress. Total soluble protein decreased with increasing stress while the specific activity of peroxidase increased with detached leaves (Todd and Yoo, 1964).

Water stress also caused a rapid increase in the ABA content of leaves of wheat (Wright and Hiron, 1969). Such responses to water stress may be due to the accumulation of ABA or to the changing ABA/cytokinin balance. Water stress and ABA can provoke effects on leaf protein synthesis which are similar to the changes that occur during senescence. Cooke et al., (1979b) reported that the loss of protein brought about by water stress was the result of reduced protein synthesis and enhanced protein degradation. However, an alternative strategy which plants may use to adapt to a water stress situation is to change its enzyme complement by synthesizing new proteins from amino acids formed by degradation of old protein (Dhindsa

and Cleland, 1975).

K treatment, using 6 day-old wheat seedlings, resulted in an increase in the level of soluble protein, after 48 h and 72 in the light. However, K treatment using old seedlings caused a slight increase in soluble protein after 48 h in the light, but there was no effect of K after 72 h. Treatment with K in the dark caused a slight increase in the soluble protein after 48 h in young seedlings. However, K had no effect on soluble protein levels after 72 h in young seedlings or after 48 h and 72 h in older seedlings. The different responses of seedlings to K may be due to the difference in developmental stages of the plant. However, roots of the wheat seedlings exhibited a declining level of soluble protein with K treatment compared to the leaves. In order to assess changes in soluble protein level occurring as a result of K treatment of wheat seedlings, studies were carried out with excised leaves and leaf segments. K treatments with excised leaves had no effect on the level of soluble protein in both light and dark incubation. Furthermore, leaf segments gave a similar pattern to excised leaves. However, there were small differences between the samples which may reflect the tissue age and the time of incubation.

Treatment with K and PEG, as a mixture, caused the level of soluble protein of wheat seedlings (6 and 10 day-old) to decline in young and old leaves in the light and dark after 48 h and 72 h. This pattern of responses was not found with excised leaves and leaf segments, which were not affected by a combination of K and PEG. Again, there were slight differences between the samples as stated earlier which may have been due to the developmental stages and the time of incubation. However, it seems more likely that the PEG solution, K treatment and a combination of both solutions did not affect either excised leaves or leaf segments, but affected the wheat seedlings as a results of interaction between the root and shoot system. However, it

has been reported that K treatment caused a 25% increase in protein level compared to control samples (Jones and Kaufman, 1971). Itai and Vaadia (1971) reported that when plants were subjected to water stress, translocation of cytokinin from the root zone decreased and the decline in protein synthesis in the leaves of these plants may be linked to fall in cytokinin levels. Cooke et al. (1980a,b) suggested that water stress, perhaps acting via hormones, affects the permeability of the tonoplast and allows the vacuolar proteolytic enzymes to interact with cytoplasmic protein.

The results in this section were not totally explainable since large varaitions were found in protein content under the various treatments. However, it can be concluded that the incubated excised whole leaf blades and segments had lower protein contents than for the leaves of intact seedlings. This pattern was seen for dark and light incubations and for the 6 and 10 day-old tissues. Root systems from the intact seedlings showed that the amount of protein declined for the 6 day-old to 10 day-old seedlings. This pattern was more pronounced than for the leaf tissue and indicated that the root tissue varied independently of the leaf tissue in regard to protein content.

CHAPTER FOUR

NUCLEIC ACIDS

4.1 Introduction

Nucleic acids are the major components of cell nuclei. They were discovered in animal tissues by Miescher (1871). The first nucleic acids isolated from plant tissues were from yeast (Altmann, 1889; Osborne and Harris, 1902).

DNA in higher plants varies in amount and type depending on the species (Flavell, 1982). The haploid DNA content of angiosperms is variable, ranging from 0.5 to over 200 pg per cell (Bennett and Smith, 1976; Rees and Hazarike, 1969) with, for example, a three-fold variation in the genus Lathyrus and ten-fold variation in the genus Crepis (Jones and Brown, 1976). Hinegardner (1976) reported that higher plants contain more DNA than is required for developmental processes. DNA in the nucleus is found in the form of chromosomes but smaller amounts are present in cellular organelles such as mitochondria and chloroplasts (Zubay, 1988). Organelle DNA has been found in different plastid types, proplastids (Edelman et al., 1964), etioplasts (Herrmann and Kowallik, 1970), chromoplasts (Herrmann, 1972), leucoplasts (Siu et al., 1976) and chloroplasts (Rose et al., 1974). Kowallik and Herrmann (1972), Gibbs et al. (1974) and Siu et al. (1976) have reported that the DNA fibrils are found in more than one region within organelles.

In higher plants, the number and size of DNA regions increases during development of the chloroplast, indicating genome amplificatation. The amount of DNA increases (Herrmann and Kowallik, 1970; Kowallik and Herrmann, 1972). Incubation of tissues in radioactive nucleotide precursors results in DNA being labelled in all chloroplasts following organelle division as noted by Rose et al. (1974) and Poss-

ingham and Rose (1976) with spinach chloroplasts; and Kadouri et al. (1975) with cucumber chloroplasts. These observations suggest that DNA is synthesized in the chloroplasts themselves. All cellular DNA synthesis is restricted to the nucleus, mitochondria and chloroplasts. Localization of DNA polymerases within these organelles show that 90% of the total DNA polymerase activity is in the nucleus (Weissbach, 1977). DNA polymerase activity has been reported to be increased prior to the onset of cell division in washed slices of sugar beet (Dunham and Cherry, 1973) and in potato tuber (Watanabe and Imaseki, 1977). DNA polymerase activity has been reported in pollen grains of Tradescantia spp. (Takats and Weaver, 1971).

It has been found that the amount of DNA per cell may affect the rate and duration of different developmental process. DNA content in higher plants is reported to be correlated with such features as cell size, size of tissues, type of organ, cell cycle time and duration of S phase, duration of meiosis, pollen maturation time and minimum generation time (Rees and Jones, 1972). DNase activities have been found associated with DNA replication, recombination and repair in bacterial systems. They are also associated with the degradation of foreign DNA in bacterial and animal cells, and in catabolic processes associated with cellular senescence (Flashman and Levings, 1981).

Different sites of DNase in plant cells have been demonstrated. These sites include chromatin (Kligman and Takats, 1975), the vacuole (Matile and Winkenbach, 1971), lysosomes (Coulomb, 1971) and the cytosol (Butcher et al., 1977). Chromatin-associated and free nuclear DNase have been reported to be associated with DNA replication in seedlings (Jenns and Bryant 1978). It has been reported that lysosomal and vacuolar enzymes serve as a defence against viral infection (Flashman and Levings, 1981). Vacuoles contain a variety of degradative enzymes in addition

to DNase. The vacuole is the primary cellular site of such activity (Flashman and Levings, 1981). It has been reported from work using thymidine incorporation, that nuclear DNA and plastid DNA are synthesized during greening and synthesis of the former precedes plastid DNA synthesis. This appears to be due to an increase in the population of chloroplasts where plastid DNA synthesis coincides with chloroplast division (Kowallik and Herrmann, 1974; Rose et al., 1975). The ratio of plastid DNA to nuclear DNA is higher in fully green pea shoots than in younger ones. It has been reported that the amount of plastid DNA in young leaves is 1.3% of the total DNA and 7.3% of the total DNA in the old green shoots (Lamppa and Bendich, 1979).

Ribosomes are known to play an important role in transforming genetic information into cellular realisation (Von Wettstein et al., 1971). They are commonly regarded as a RNA-protein complexes through which mRNA passes, with consequent translation of the genetic code into polypeptides, by sequential amino acid assembly (Davies and Larkins, 1980). The rRNA serves to provide a template for ribosomal protein assembly since partial destruction of rRNA need not mean the loss of ribosomal function (Furano and Harris, 1971).

Ribosomes are made up of two nucleoprotein subunits. The subunits contain between 30 and 50% (by weight) protein, the remainder being RNA and a minor amount of inorganic molecules (Davies and Larkins, 1980). The classification of ribosomes is based on their sedimentation coefficients. Ribosomes (70S) occur in prokaryotic organisms such as bacteria and blue-green algae, and in the chloroplasts of plants. Ribosomes (80S) are reported to be in the cytosol of eukaryotic cells (Loening, 1968; Stutz 1976; Davies and Larkins, 1980). These are more larger and more complex than 70S ribosomes of bacteria (Wool and Stoffer, 1974). Bacteria have only one genome and produce just one ribosomal 70S.

In contrast, animals have two genomes with 70S ribosomes in the mitochondria and 80S ribosomes in the cytoplasm. Green plants have a third genome and an additional ribosome of the 70S type in the chloroplast (Loening, 1968; Stutz, 1976). The RNA component of 80S ribosomes is synthesized directly from a DNA template in the fibriller region of the nucleus, whereas the ribosomal proteins are made on cytoplasmic polyribosomes and are transported into the nucleus for assembly (Warner et al., 1973). In eukaryotes, ribosomal RNA is synthesised as a large precursor molecule by a specific enzyme, RNA polymerase I (Perry, 1976; Hadjiolov, 1977; Krakow and Kumar, 1977). Plastids have their own complement of ribosomes which belong to the prokaryotic type (as mentioned before) and are different in their functional and physiological properties to those of 80S type (Leaver and Pope, 1977). In photosynthetic tissue, plastid ribosomes comprise about 50% of the total cellular ribosome complement and are the main contributors to cellular protein biosynthesis (Ellis, 1976).

Internal and the external factors (as mentioned in Chapters 1, 2 and 3) have their effect on cellular components including nucleic acids. It has been reported that the levels of NA found in a particular tissue are related to the age of the tissue. A decrease in leaf NA with age has been noted in tomato leaves and the percentage of RNA decreased as the leaves aged (Gates and Bonner, 1959). Holden (1952) showed that the DNA and RNA per dry weight of tobacco leaves were higher in the youngest leaves than in the oldest one.

It was suggested that the cotyledons of tobacco leaves contained storage NA which were broken down during germination and transported to the growing parts where resynthesis occurred (Smillie and Krothov, 1961). Similar results have been reported by Barker and Douglas (1960), working with pea seeds. Mothes et al. (1958)

found that during the growth of Nicotiana rustica leaves the levels of NA started to increase, followed by a decrease in the levels, which eventally attained a fairly constant level. Similar findings were reported by Holmes et al, (1955) using bean leaves, and Samuels (1959), using pea roots. Osawa and Oota (1953) and Oota and Osawa (1954) have reported that, during germination of the bean Vigna sesquipedalis, a rapid decrease in the NA levels of the cotyledon occurred. This was accompanied by a parallel increase in various parts of the growing seedlings. The ageing of leaves is characterized by a gradual decrease in metabolic activity, manifesting itself in yellowing and degradation of NA (Wollgiehn, 1967). The levels of RNA subjected to degradation during ageing of tobacco leaves are strongly dependent on a supply of nutrients. However, after excision, the young tobacco leaves aged more slowly than old ones (Wollgiehn, 1967). It has been reported that during the initial stages of germination in the peanut, there is an increase in the levels of RNA and DNA due to enzymic changes during the developmental process (Aldana et al., 1972).

Plant growth regulators (especially cytokinins) have different effects on NA. It has been reported that cykokinin enhanced RNA in tobacco leaves (Partheir and Wollgiehn, 1961). Similar results have been reported by Srivastava (1967), using barley leaves. Cytokinin is reported to enhance endogenous polymerase activity (Johnson and Kende, 1971). It has been noted that cytokinin induced increases in nuclear RNA and nuclear labelling with RNA precursors (Guttman, 1957; Olszewka, 1959; Jensen et al., 1964). Carpenter and Cherry (1966), using peanut cotyledons, reported that cytokinin increased the levels of all RNA species within 2 h of hormone application. Similar findings of enhanced RNA by cytokinin have been reported by Zwan (1973) and Erismann and Fankhauser (1967), using Lemna spp. It has been found that cytokinin promotes the activity of nuclear polymerase 1 that catalyzes the transcription

of ribosomal RNA (Schneider et al., 1978; Romanko et al., 1978). Mikulovick et al. (1978) reported that cytokinin promoted RNA synthesis in the greening tissues. Similar results have been found by Dyer and Osborne (1971) and Grierson et al. (1977). Cytokinins have been shown to decrease RNase levels in intact leaves (Fletcher, 1969).

Light has an important role in the physiological and biochemical events which occur during leaf development and greening (Dale and Murray, 1968). It has been demonstrated that the illumination of etiolated tissues elicits an increase in the total RNA (Bogorad, 1970; Tester, 1977). Harel and Bogorad (1973) reported that the activity of chloroplast RNA polymerase increased after illumination of etiolated maize leaves. Similarly, Apel and Bogorad (1976) reported that the activity of RNA polymerase increased from three to four-fold within 16 h of illuminating etiolated maize plastids. They also reported that light enhanced activity of maize plastid DNA-dependent RNA polymerase. It has been noted that light increased the amount of chloroplast and cytoplasmic ribosomal RNA, and that higher levels occured in plastid rRNA (Roussaux et al., 1976; Mikulovich, 1978). Similar results have been reported by Harel and Bogorad (1973), using green maize leaves. They suggested that light enhanced chloroplast rRNA synthesis came from the elevated activity of plastid RNA polymerase which has been reported to occur immediately after the illumination of etiolated maize leaves (Bogorad, 1976; Harel and Bogorad, 1973).

In addition to the normal enlargement and greening of etiolated tissues following illumination, it has been shown that there is a consistent increase in the NA levels. This increase in RNA could arise as a result of increased cytoplasmic and chloroplast synthesis (Rhodes and Yemm, 1966). It has been reported that the increase in RNA levels may accompany the unrolling and greening process (Bogorad and Jacobson, 1964). Similar results have been noted in barley leaves (Bogorad, 1967;

Kirk and Tilney-Bassett, 1967).

One of the major effects of illumination on etiolated pumpkin cotyledons is an accumulation of ribosomal RNA (Wollgiehn and Parthier, 1980; Mikulovich, 1978). Poulson and Beevers (1970) reported that the capacity of RNA synthesis was increased in response to light, with barley leaf segments. They also reported that light stimulated RNA polymerase activity in maize leaves. It has been reported that white and red light have an effect on morphological and biochemical changes in cellular components, one effects being increased soluble RNA polymerase activity. Pearson and Wareing (1970) noted an increase in polysomes with white and red light using wheat leaves. Similar results have been reported by Thien and Schopfer (1975), using mustard cotyledons. They found an increase in cytoplasmic and plastid rRNA in the presence of white or red light.

Many investigations have been carried out on structural aspects of water stress on development and metabolic processes (Kessler, 1961). It has been reported that the most consistent response of a plant cell to water stress is an increase in RNase activity (Diener, 1961, Dove, 1967, 1971; Arad et al., 1973). It has been suggested that the increase in RNase activity parallels the increase in water stress, due to an increase in the concentration of ABA (Arad and Richmond, 1970). Premecz et al. (1977) reported that the increase in RNase level is due to enzyme protein synthesis. Reduction in RNA synthesis rates during the imposition of water stress has been reported for corn seedlings (Maranvill and Paulson, 1972), and for the desert plant Anastatica hierochuntica where recovery occurred within 12 h of rewatering after severe water stress (Hartung, 1974). Nir et al. (1970) found that water stress-induced changes to cells caused condensation of DNA in the nucleus thus blocking messenger RNA synthesis and, ultimately, protein synthesis. Henckel et al. (1967),

Henckel (1970) and Blekhman (1977) demonstrated that there was an increase in ribonuclease activity in a variety of plants subjected to water stress. Hsiao (1973) reported that moderate water stress probably had little effect on NA composition, and severe deficiency of adenosine triphosphate (ATP) might slow NA synthesis and prevent genome replication. In stressed tissue, RNase activity increased and may have inhibited accumulation of RNA (Hsaio 1970).

Hsaio (1970) found that water stress caused a reversible decrease in NA in wheat leaves and accumulation of intermediate products of NA metabolism. In agreement with other workers investigating wheat Todd and Basler (1965) and Stutte and Todd (1969) reported that more severe water stress caused irreversible NA decomposition. Morilla et al. (1973) noted that the increase in ribonuclease followed polysome level decline during water stress with corn seedlings. Henckel et al., (1967) reported that water stress caused the disappearance of polysomes in corn and bean leaves and the appearance of free ribosomes. It has been reported that stressed tomato leaves (Lycopersicon esculentum) retain the ability to incorporate ^{32}P into RNA, but the rate of destruction of RNA is increased (Gates and Bonner, 1959). When barley plants were exposed to water stress, the RNA levels of developing pollen decreased (Simonovitch 1963). Wyen et al., (1969), using Avena spp. leaves, reported that the decrease in RNA was associated with increased RNase activity and this was associated with injury and senescence. Kessler (1961) noted that the decline in RNA levels during water stress was due to impaired RNA synthesis or increased hydrolysis. Water stress impaired the NA system which is intimately connected with protein synthesis (Cosperson, 1950).

The major aim of the work presented in this Chapter was to investigate the level of NA, particularly RNA, in the development and the greening process of wheat

leaves where the capacity to accumulate chlorophyll is altered with the ageing of the etiolated tissue. Particular attention was paid to the fractionation of the NA with a view to distinguishing which ribosomal fractions (if any) changed specifically, i.e the chloroplastic or cytoplasmic component. The effect of water stress on this process was also investigated in order to elucidate what effect environmental factors, other than light, might have. Again the aim was to see if, given that chlorophyll accumulation was impaired, the chloroplast specific ribosomal RNA levels were also altered or if the effect was generally on all heavy molecular weight ribosomal RNA fractions.

4.2 MATERIALS AND METHODS

In all cases of NA extraction 500 mg of leaf tissue was used.

4.2.1 Material

4.2.1.1 Plant material: as described in General Materials and Methods.

4.2.2 Methods

The method used was that of Solymosy et al. (1968,1970) and modified by Chaffey (1983).

4.2.2.1 Extraction of NA

Leaf tissue of up to 0.5 g was placed in a mortar and ground to a fine powder in the presence of liquid nitrogen and treated sequentially as follows:-

a. To the homogenate was added 0.32 ml diethylpyrocarbonate (DEP), 0.88 ml of 10% sodium dodecylsulphate (SDS) (100 g. l^{-1}) and 4.4 ml extraction buffer (100 mM Tris/HCl, 53 mM sodium acetate, 8.8 mM magnesium acetate, pH 7.2). The homogenate was mixed thoroughly then poured into a centrifuge tube.

b. A further 4.4 ml extraction buffer was added to the mortar, the washing was combined with the homogenate from (a) above.

c. NaCl (0.25 g) was added to the combined homogenates from (a) and (b) above and after it was dissolved the solution was centrifuged in a bench top centrifuge at 2,000 rpm for 10 min. The supernatant was decanted into a centrifuge tube containing 12 ml (100%) ethanol, the top sealed with parafilm and the mixture then stored at -5^{o}C.

d. The ethanolic NA solution from (c) was centrifuged, the supernatant discarded

and precipitate allowed to air dry before being dissolved in 1.5 ml of (E) buffer solution (40 mM tris base; 20 mM sodium acetate; 2.5 mM magnesium acetate, pH 7.2).

e. The NA solution was further centrifuged if necessary and supernatant retained for NA determination.

4.2.2.2 NA estimation and fractionation

The method of Chaffey (1983) was used with minor alterations. Acrylamide and bis-acrylamide were dissolved in water to make a stock solution of 15% w/v and 0.75% w/v, respectively. The 10% (w/v) ammonium persulphate solution was made up fresh each time. Buffer (3E) was prepared using:

trizma base 120 m M

sodium acetate 60 m M

magnesium acetate 7.5 m M

The pH was adjusted to 7.2 with M HCl. A gel running buffer (3E) was prepared by diluting (3E) 1:2 with distilled water into which was dissolved 2 g SDS. l^{-1}.

4.2.2.3 Gel preparation

Acrylamide gels (Strength 2.6%) were made using the following reagents:-

stock acrylamide (4.33 ml)

(3E) buffer (8.32 ml)

distilled water (12.11 ml)

NNN'N'-tetramethylethylene diamine (Temed) (0.010 ml)

ammonium persulphate (0.10 ml)

The acrylamide solution, buffer, water and Temed were mixed in a beaker. Ammonium persulphate solution was added and quantities of the mixture pipetted to a depth of 8 cm in 0.45 cm diam. plexiglass gel tubes which had their lower ends sealed with dialysis membrane held with a rubber ring. Closed rubber tubes were fitted over the membrane to form an air seal to prevent the solution passing through the membrane. Polymerisation was completed in 20 minutes, after which the air seals were removed and the gel transferred to an electrophoresis tank.

4.2.2.4 Electrophoresis

This was carried out at room temperature (21°C). The gels were pre-run at 6 mA. gel $^{-1}$ for a 30 min. prior to loading the samples, to remove free acrylamide, ammonium persulphate and other impurities (Poulson and Beevers, 1970). RNA (10 to 20 μg in 15 μl of (E) buffer (to which had been added a small quantity of solid sucrose) was loaded onto each gel

Electrophoresis was perfomed for $3\frac{1}{2}$ h at 6 mA/gel and 50 volts in plastic electrophoresis tanks. At the end of this period, the dialysis membrane was removed and the gels gently blown into a Petri-dish and soaked in 7% acetic acid for 12 h to remove any strong ultra-violet light-absorbing material present at the top of the gel. Gels were scanned in a Joyce Loebl 400 linked to Joyce Loebel D7 power supply and potentiometric recorder on which the peaks absorbance of the RNA were obtained. The peak heights and the ratio between total cytoplasm and total chloroplast were recorded from traces by measuring the area under the peaks by manual integration.

4.2.2.5 Quantitative NA determination

NA solution (0.1 ml) was made up to 3.0 ml with distilled water and scanned in an Ultrospec (400 LKB) Biochrom, using distilled water as a blank. NA concen-

tration was calculated from the following equation of Tester and Dure (1966).

$22\ A_{260} - A_{290} = 1\ mg\ ml^{-1}$ nucleic acid.

Whilst fractionation into discrete components was possible on the gels no actual quantitative yield could be obtained for each fraction.

4.3 RESULTS

4.3.1 Total NA levels during greening of the first leaf of wheat seedlings

In preliminary experiments, first leaves of wheat seedlings were segmented into three segments (tip, middle and base) after the seedlings had been exposed to light for 72 h following growth in the dark for 10 days. Total NA were extracted and the levels estimated for each segments. The data in Fig. 4.1 show that the distribution of NA was very similar for all segments. The middle segments contained the highest level of NA followed by the tip segments. The lowest level of NA was in the basal segments. These total NA levels along the length of greening leaves did not follow the same pattern as seen for chlorophyll (the greening process from the tip to the base).

This pattern was further investigated in relation to the treatment applied with factors such age, K and water stress. The first leaves of whole seedlings (6 or 10 day-old) which had not been exposed to light were used. The level of NA in the leaves was estimated for seedlings kept in the dark and the light for 48 h and 72 h using both young and old tissue.

The level of NA accumulated in the tissues, which were incubated for 48 h and 72 h in the light and the dark, are shown in Table 4.1 and 4.2, respectively. There was an increase in the level of NA in young tissue after 72 h of incubation in water. In contrast, there was no change in the NA level in the older tissue over the period of the experiment (Table 4.1). Samples incubated in the dark showed higher levels of NA after 48 h on the young tissue. However, after 72 h the level of NA declined in the tissue in the light (Table 4.1). The capacity of the older seedlings to accumulate NA was less than that of the younger seedlings.

Figure 4.1

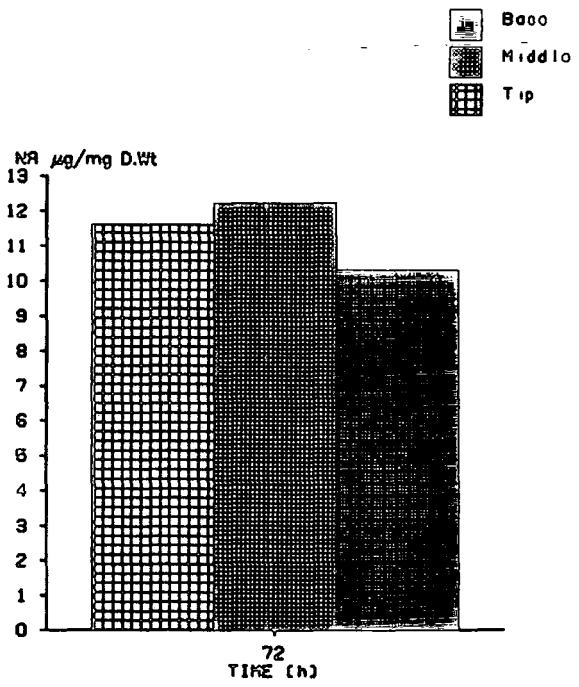


Table 4.1

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	10.1	13.0	12.2	13.4	16.9	14.6
	72	17.0	16.4	15.9	20.2	18.1	15.6
10	48	10.4	11.5	9.2	10.5	11.7	11.9
	72	10.2	9.3	10.9	9.9	9.5	12.0

Table 4.2

Tissue age (day)	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	13.6	10.6	10.9	11.4	12.1	14.6
	72	10.8	13.9	13.1	17.4	11.2	14.8
10	48	9.9	9.1	7.4	8.7	9.9	7.1
	72	10.3	11.9	9.8	7.9	11.4	10.7

The effect of water stress on the NA content of the tissue was investigated by treating seedlings for 48 h and 72 h with either a low or a high concentration of PEG prior to extraction. The level of NA increased slightly in young tissue after 48 h of incubation in the low PEG concentration, but declined after 72 h (Table 4.1). A similar response was found for the tissue incubated in the dark (Table 4.2). This indicates that the response of NA accumulation to PEG was not related directly to the greening of tissue. The level of NA increased in the high PEG concentration in young tissue during the period of 48 h to 72 h of incubation in the light and in the dark. However, there appeared to be no effect of PEG on older tissue over the sampling period (Table 4.1 and 4.2).

The results of the effect of K on 6 day-old wheat seedlings which were incubated in the light or dark for 48 and 72 h are shown in Table 4.1 and 4.2. It appears that the levels of NA were increased slightly in young tissue after 48 h of incubation in both the light and the dark compared to water controls. There was a slight decrease in older tissue after 72 h in the light, and a slight increase in levels in older tissue incubated in the dark for 72 h. These effects were found to be variable and there was no consistent effect in any of the treatments.

The effect of K as a potential inhibitor of stress imposed by PEG on the level of NA was also investigated and the results are shown in Table 4.1 and 4.2. For the light investigations, it was noted that the level of NA was higher in the young tissue after 48 h incubation with both PEG concentrations and K in comparison to the PEG or K alone. The level of NA was higher after 72 h incubation with the low PEG concentration and K, and slightly decreased in the same period with the higher PEG concentration and K. There was no effect of the combined treatments (K and PEG) in the older tissue incubated in the light. The level of NA in the tissue incubated

in the dark with similar combined treatments is shown in Table 4.2. There was no consistent effect of PEG and K on NA levels. There were increases and decreases in levels of NA in treated and control-plants. In the young tissue, it appeared that a combination of PEG and K abolished the increase in levels of NA over the sampling period.

It was noted that the various treatments had no effect on NA levels of root systems (Table 4.3 and 4.4). However, for the 6 day-old dark-grown tissue, the overall level of NA was higher than for light grown tissue, at both times of incubation. The 10 day-old tissue gave similar levels of NA in the roots of the light and dark-grown seedlings. It appears that the effect of water stress was apparent not on the root system but on the leaves.

The effect of water stress on leaves was studied further using excised leaves and leaf segments. Seedlings were grown in the dark for either 6 or 10 days-old, prior to excision of the leaves before being transferred to the light. Tissue from both excised leaves and leaf segments (tip, middle and base) were treated in the same way as the seedlings. It was noted that levels of NA in excised leaves were generally higher when incubated in the dark compared with the light (Table 4.5 and 4.6). These effects were apparent for leaves derived from 6 or 10 day-old dark-grown seedlings following incubation for 48 h and 72 h in the light.

It appeared that K, PEG treatment and a combination of PEG and K had no consistent effect on the levels of the NA extracted from the excised leaves (Table 4.5 and 4.6). Analysis of the total NA levels extracted from leaf segments which had been incubated in the same way as excised leaves are shown in Table 4.7 and 4.8. Once again, no consistent effect of the treatment was seen on the total NA for either time of treatment.

Table 4.3

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	3.9	3.8	3.4	3.2	3.3	2.9
	72	4.5	3.2	3.9	4.6	4.6	3.6
10	48	3.9	4.4	3.6	3.7	4.3	4.1
	72	3.2	3.7	4.3	3.8	4.3	3.8

Table 4.4

Tissue age (day)	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	5.4	4.8	6.4	6.3	5.7	5.9
	72	5.7	6.3	6.1	5.6	5.4	5.7
10	48	3.8	4.0	4.3	3.3	4.2	4.7
	72	5.1	4.3	5.4	3.5	5.4	5.1

Table 4.5

Tissue age (day)	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	6.9	7.2	6.8	5.5	6.2	3.8
	72	6.4	6.3	6.9	6.5	6.6	7.8
10	48	7.7	8.0	7.2	6.9	6.0	6.4
	72	6.9	6.5	8.0	7.2	7.4	9.1

Table 4.6

Tissue age (day)	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
6	48	10.4	8.7	8.7	8.5	8.3	8.5
	72	7.3	10.7	8.9	8.9	10.5	8.7
10	48	11.1	8.8	9.3	7.9	8.5	6.5
	72	9.3	13.0	9.3	7.2	11.8	9.1

Table 4.7

Leaf segments	Time in light (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
Tip	48	8.6	11.7	7.3	8.5	6.8	7.6
	72	8.9	10.5	8.5	8.5	9.2	8.2
Middle	48	9.9	8.9	9.3	8.4	8.6	7.1
	10	8.2	7.9	9.2	6.9	9.8	8.1
Base	48	8.9	10.0	9.0	8.6	8.7	7.1
	72	12.5	12.4	7.9	8.2	8.1	10.5

Table 4.8

Leaf segments	Time in dark (h)	Treatment					
		H2O	K	-5 PEG	-10 PEG	-5 PEG + K	-10 PEG + K
Tip	48	8.1	9.4	8.6	7.8	9.8	9.7
	72	5.1	6.0	6.4	6.1	5.2	3.7
Middle	48	8.7	9.2	7.9	6.6	7.8	7.4
	10	8.4	10.9	7.4	6.1	7.7	7.9
Base	48	10.3	10.8	8.5	9.1	9.0	8.8
	72	7.4	10.3	6.7	7.0	6.7	7.0

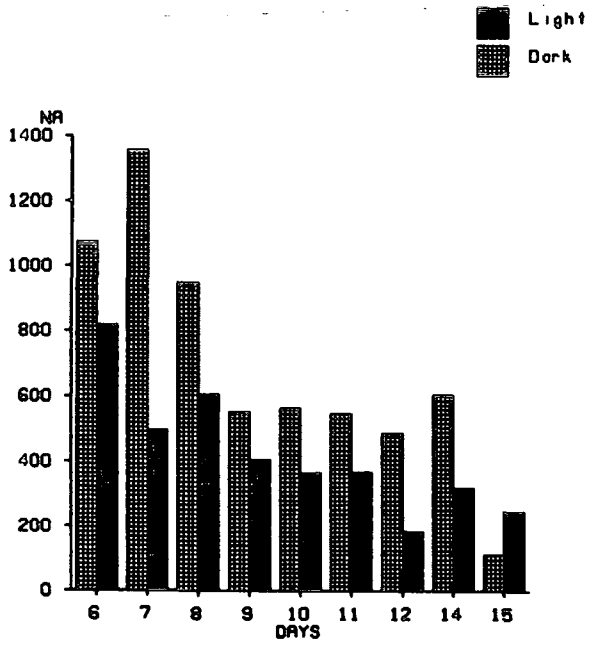
It appears that the effect of treatment on the leaf tissue was most pronounced when intact seedlings were used.

In view of the effect of light treatment (increases and decreases in levels of NA in treated and control plants respectively) on total NA in whole seedlings, a further set of investigations were carried out. Incubations were for longer periods of time and the NA was fractionated by polyacrylamide gel electrophoresis (PAGE). The total NA was estimated in segmented leaves (tip, middle and base) after incubation of the seedlings. Wheat seedlings were grown for 5 days in the dark prior to transfer to the light or kept in the dark. Incubation was for up to 17 days, with the first extractions being made from 6 days. Total NA was extracted at day 6 to 15. The total NA levels extracted from light and dark incubated first leaves of seedlings are shown in Fig. 4.2, 4.3 and 4.4 for tip, middle and basal segments, respectively. There was a general decline in NA during the time of incubation from 6 to 15 days, for tip segments incubated in both light and dark (Fig. 4.2). However, the level of NA was higher in tissue incubated in the dark compared to tissue incubated in the light. Similar results were found for middle segments (Fig. 4.3). The levels of NA in the basal segments were less consistent. In general, they were higher in tissue incubated in the dark, but decline were not apparent (Fig. 4.4). This may be a reflection of the younger age of this tissue where the NA levels were more stable and less likely to change.

The data in Fig. 4.5 show the levels of NA in segments of leaves taken from whole seedlings which were incubated for extended periods of time in the light. The level of NA in the tip segments was variable, but a decline was apparent by the end of the incubation. In the middle segments, the level of NA was higher with a general decline during the period of incubation. The basal segments showed a decline from day 13 for the level of total NA.

In order to investigate the effect of extended periods of incubation in the

Figure 4.2


Light
Dark
NA
1400
1200
1000
800
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6
7
8
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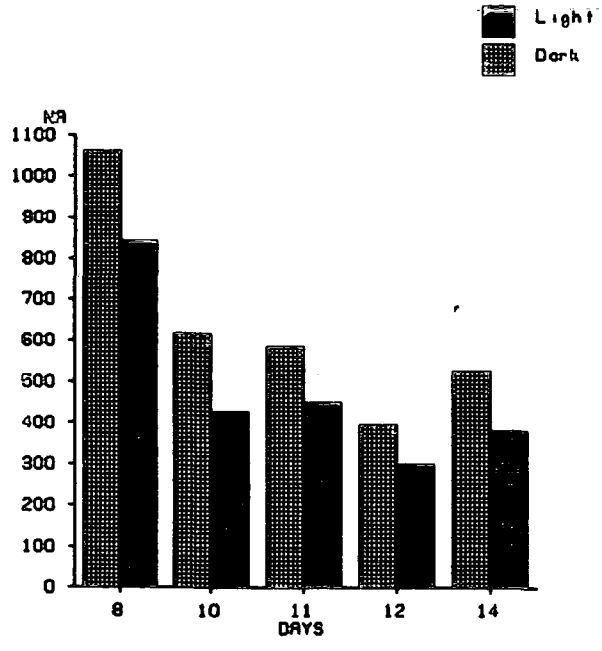
DAYS

Figure 4.3


Light
Dark
NCR
1100
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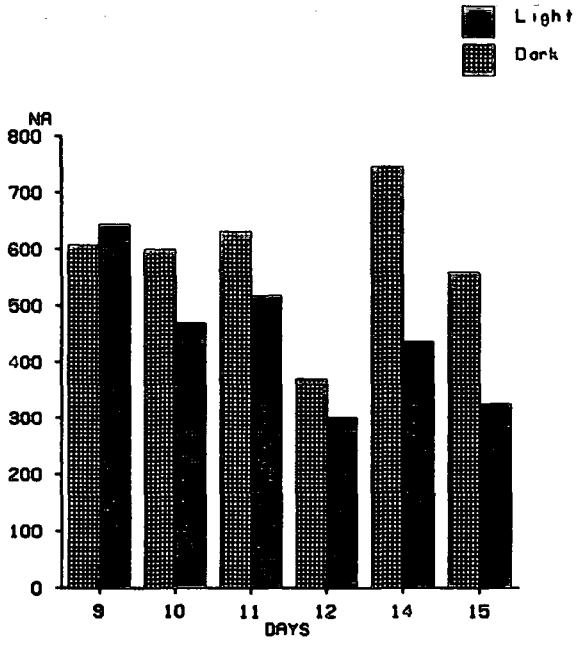
14
DAYS

Figure 4.4


Light
Dark
NA
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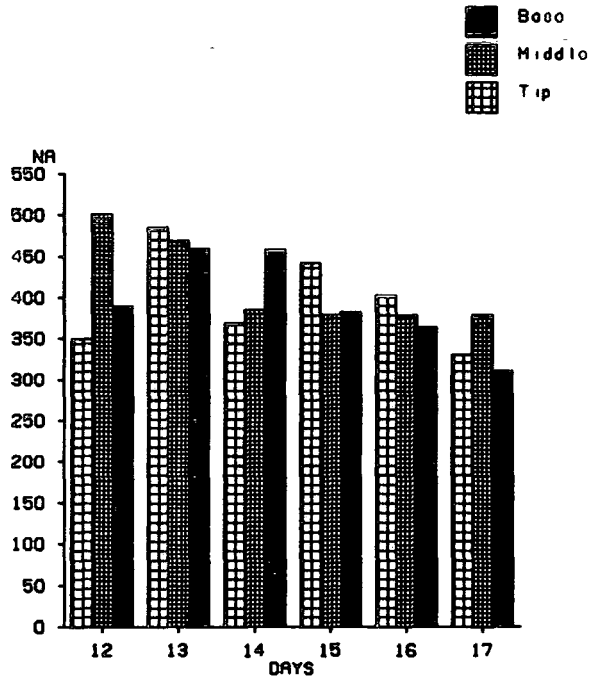
15
DAYS

Figure 4.5


Base
Middle
Tip
NA
550
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250
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100
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12
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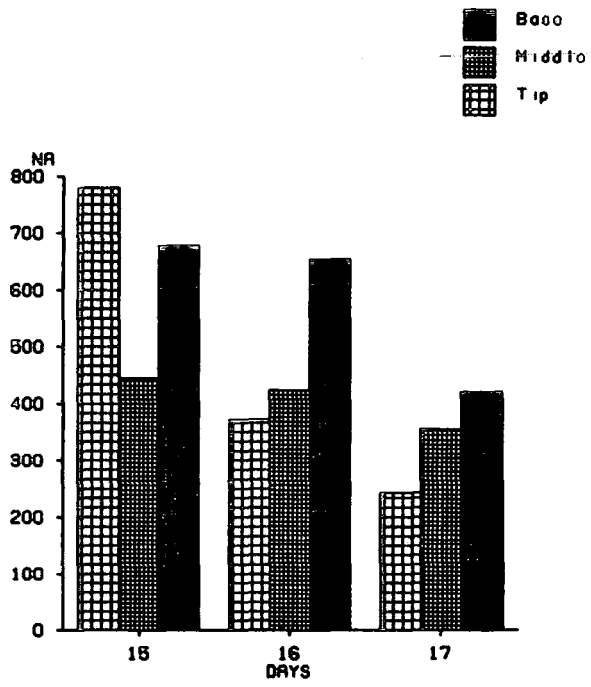
17
DAYS

dark on the total NA content of leaves and on the capacity to accumulate RNA when subseqently transferred to light after these periods, wheat seedlings were kept in the dark for 14 days prior to their exposure to light for 3 days. First leaves of seedlings were segmented into three segments as described before, then total NA was extracted and estimated. The data in Fig. 4.6 show that the level of NA was maintained during the dark incubation and was higher in the tip segments. During incubation in the light, a decline in NA was seen with the lowest level at day 17. However, the level of total NA in both middle and basal segments tended also to decrease with time. The level of NA was higher in the basal segments than in the other segments by day 17. This was probably a reflection of the younger age of these tissues.

Figure 4.6


Base
Middle
Tip
NA
800
700
600
500
400
300
200
100
0
15
16
17
DAYS

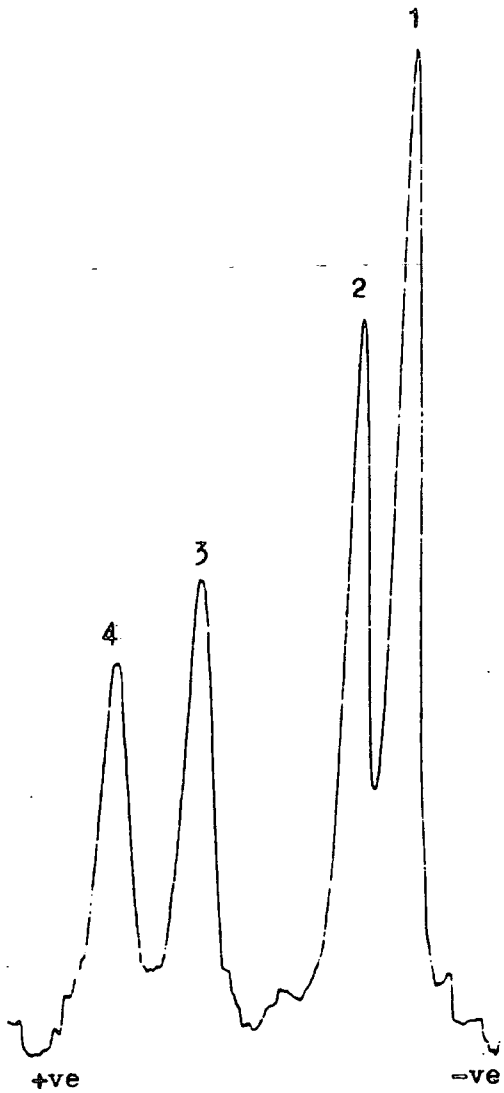
4.3.2 Fractionation of NA during greening of wheat leaves

Total NA extractions gave some indications of the changes which occurred in cellular components during greening and development. In order to investigate this further, specific fractions of RNA, particularly chloroplast RNA components were examined. Cytoplasmic and chloroplastic RNA fractionation was performed by PAGE. Gels were prepared and the RNA fractionated as described in Chapter 4.2 (Materials and Methods).

The extraction of NA was a gross method and included RNA and DNA although the vast bulk of material was RNA. Fig. 4.7 shows the fractionation of the heavy ribosomal RNA components from the cytoplasm and chloroplast. DNA, however, did not appear on the gels. The peaks designated 1, 2, 3 and 4 represent (28S) cytoplasmic RNA with an approximate molecular weight of 1.38 x 10^6 Daltons, (23S) chloroplast RNA with an approximate molecular weight 1.1 x 10^6 Daltons, (18S) cytoplamic RNA with an approximate molecular weight 0.69 x 10^6 Daltons, and (16S) chloroplastic RNA with molecular weight 0.53 x 10^6 Daltons respectively.

The data in Fig. 4.8 show the PAGE traces for segmented dark-grown leaves. All components are present in the fractionation for tip, middle and base. It appears, therefore, that all the heavy molecular weight ribosomal RNA components are maintained at high levels, particularly the chloroplast components, even in the tip which has been shown to accumulate only low levels of chlorophyll when exposed to light. The data in Fig. 4.9 show the traces for light and dark-grown 10 day-old leaves, for the middle segments as an example. All fractions of RNA were present, but the chloroplastic RNA components were higher in the light than in the dark.

The leaf tip did not accumulate high levels of chlorophyll after the transfer


1
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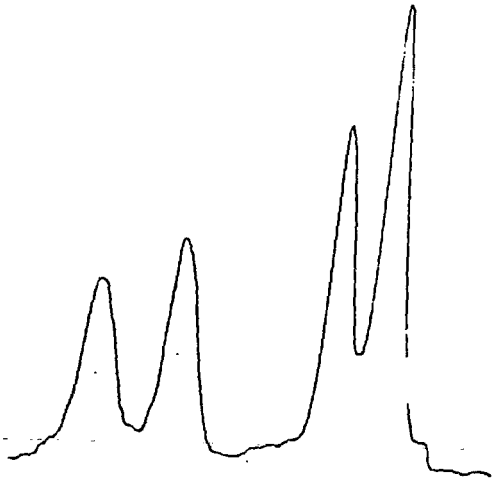

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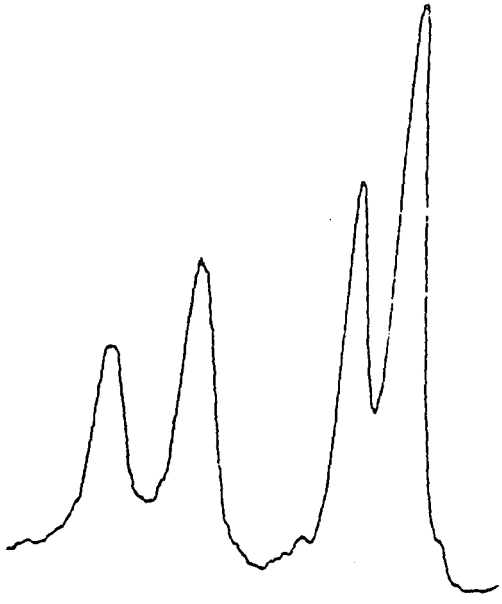

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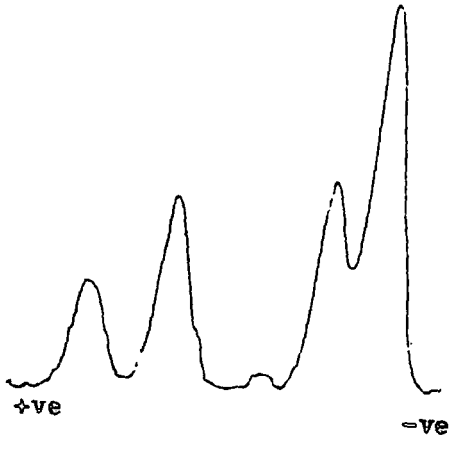

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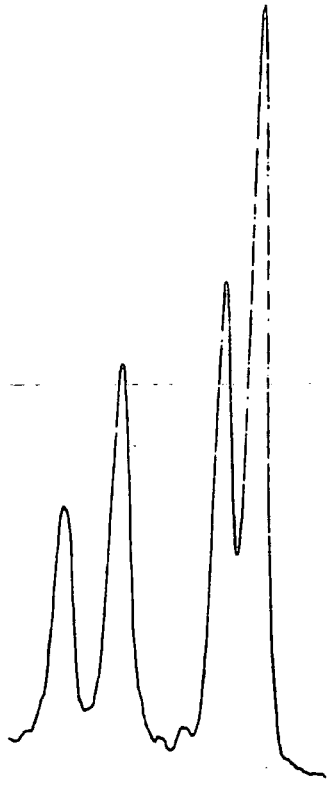

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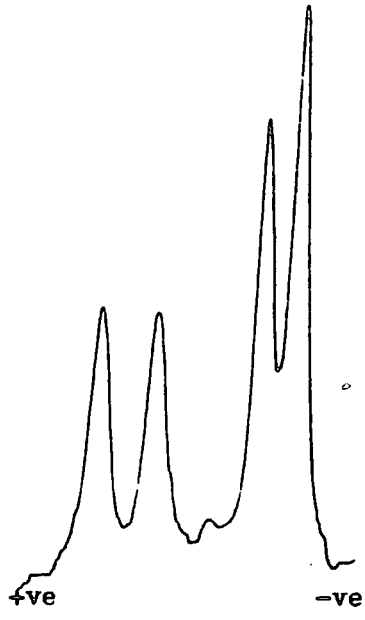

of the leaf to the light. Therefore, it was investigated which of the components of RNA were present, and also, if any of these were specifically lost or changed during exposure to light. In order to do this, seedlings were grown in trays as described in General Materials and Methods for 4 days in the dark before they were transferred to the light growth room for various periods of time. Other seedlings were kept in the dark for the same period of time as the samples which were incubated in the light. Fractionation of the RNA was started from day 6 until day 15 for both dark and light grown seedlings.

In addition, the effect on NA fractions in tissue kept in the dark for prolonged periods prior to illumination was investigated. To do this seedlings were transferred from the dark at 11 days and 14 days and were allowed to grow in the light for up to 17 days. This investigation was necessary in order to follow the pattern of development and to get a full picture of any changes in the young tissue before it reached the senescence stages. The old tissues were used to see if the same pattern of development of RNA was maintained, when they were kept in the light and the dark from the early stage of development or after they were kept for long periods of time in the dark (11 and 14 days) prior to being transferred to light.

Fig. 4.10 a–i show the traces of fractionation of ribosomal RNA from leaf tip segments which were incubated in both dark and light from day 6 until day 15. In all cases the chloroplastic RNA component was present from day 6 until day 15. All four components of cytoplasm and chloroplast were present in light and dark. However, it was noted that more chloroplastic RNA was present in the light than in the dark. All fractions were maintained in the dark with no apparent loss of chloroplastic RNA even up to 15 days of incubation. However, it was noted that at this time there was a loss of definition of components which indicated that RNA degradation had started.

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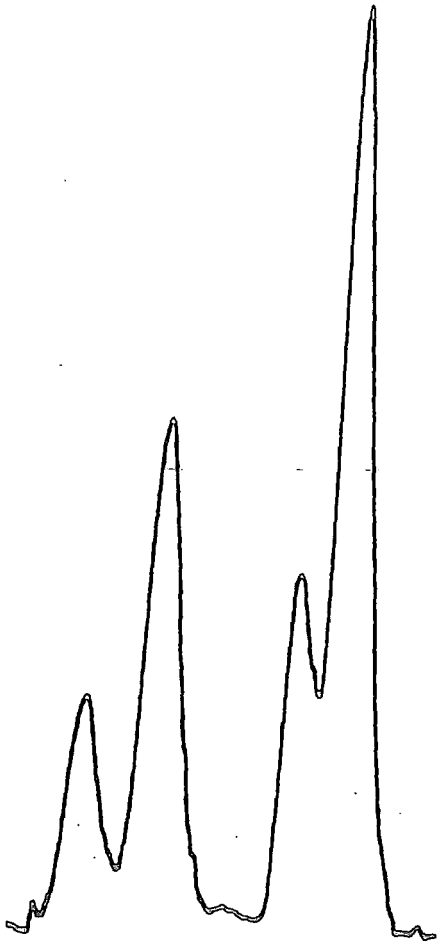

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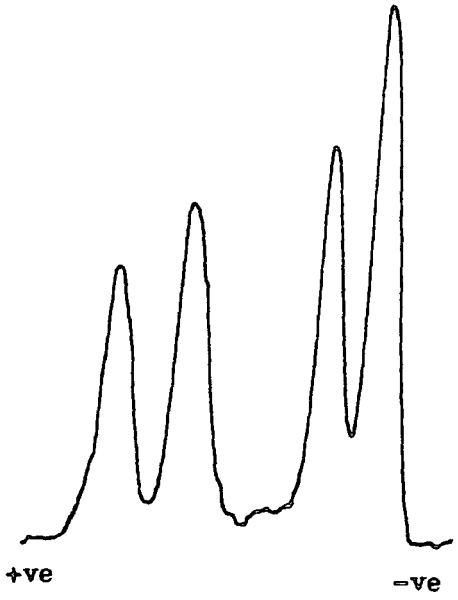

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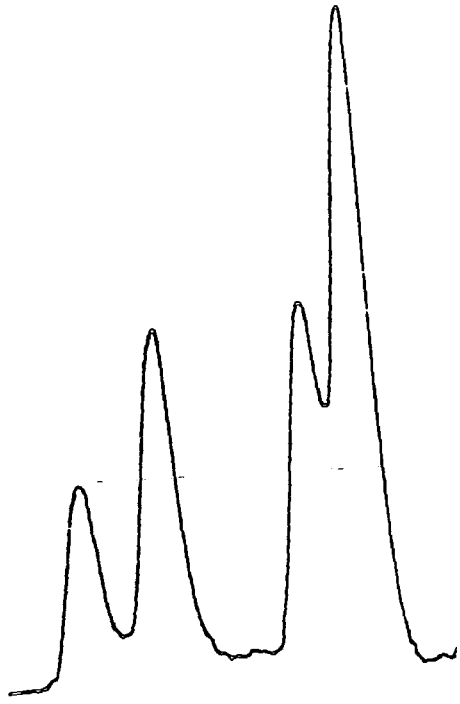

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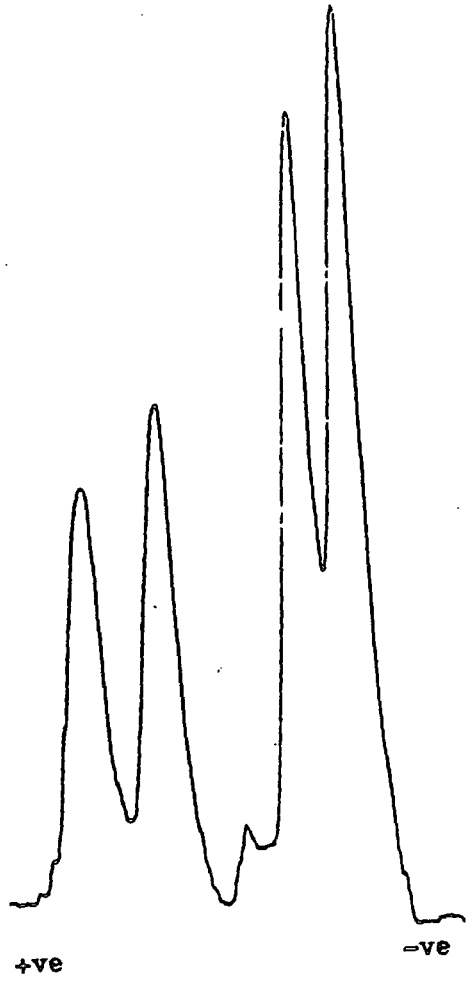

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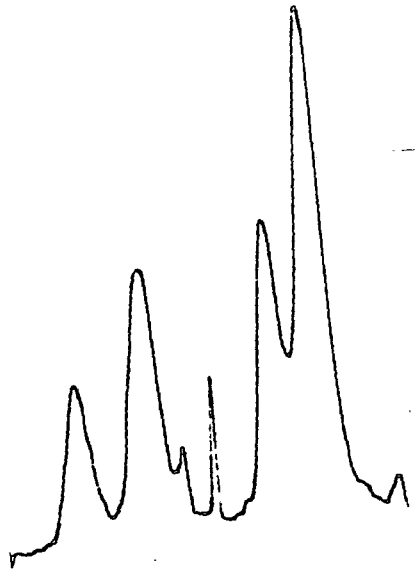

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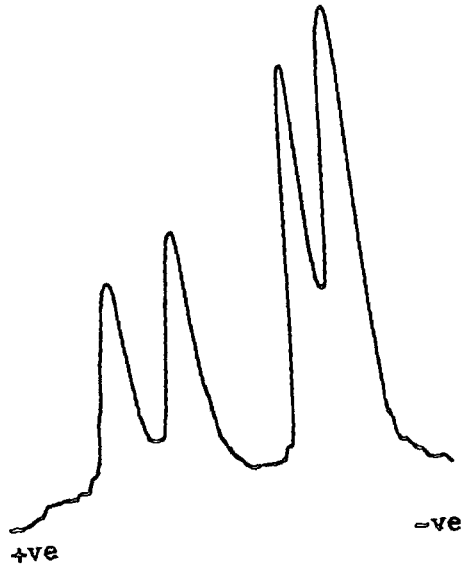

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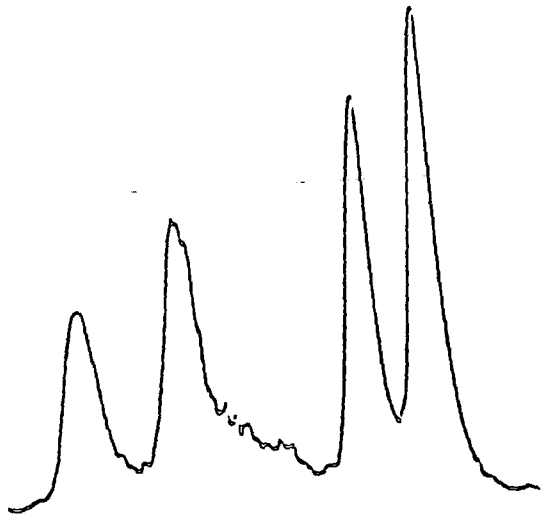

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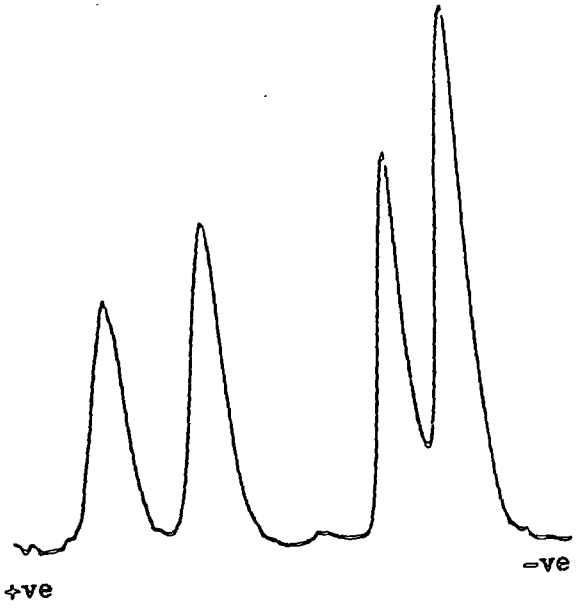

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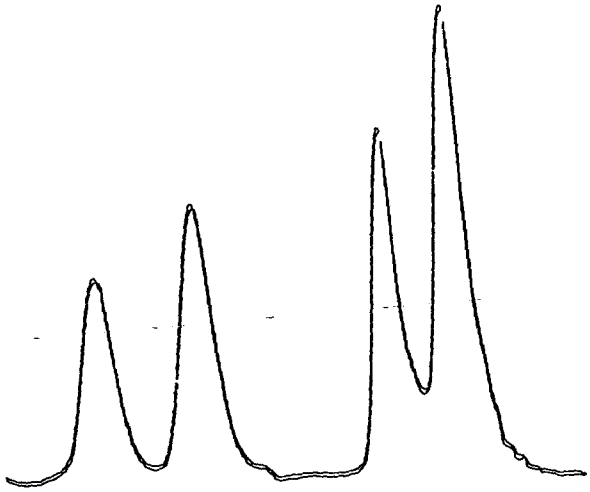

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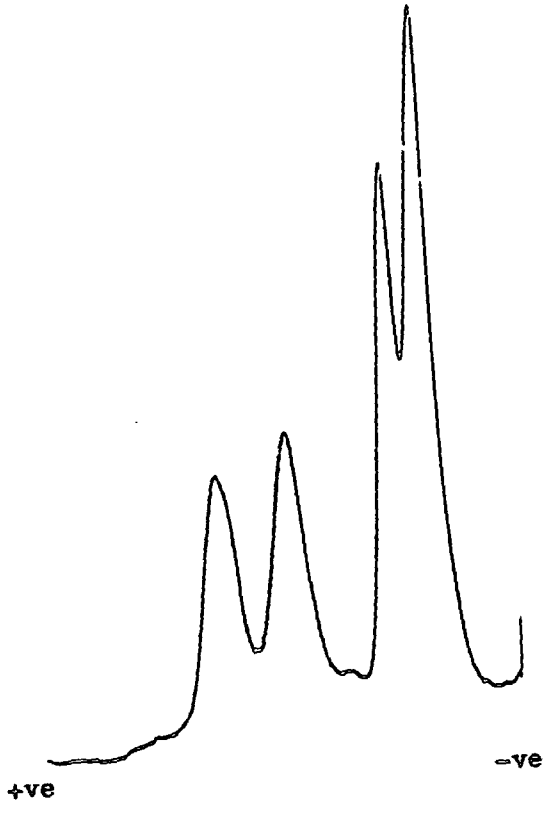

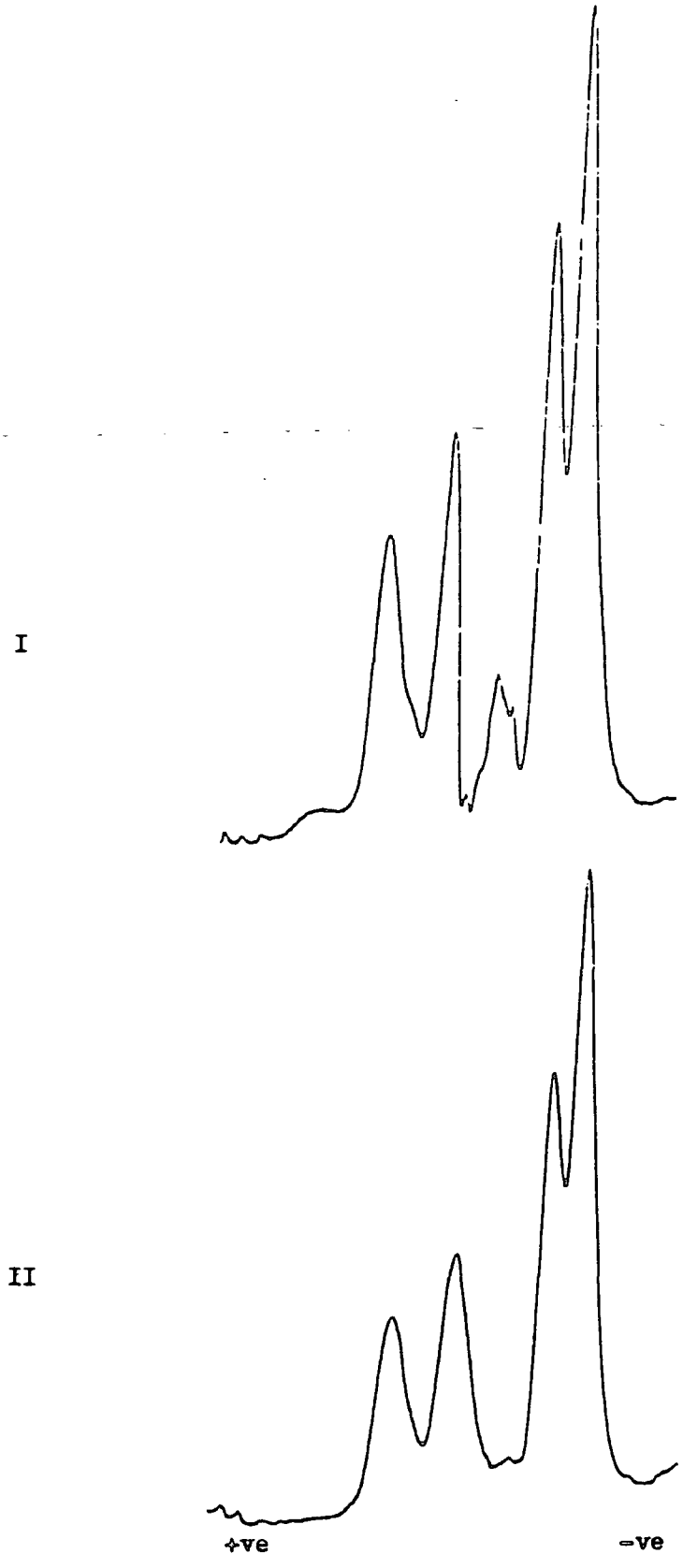
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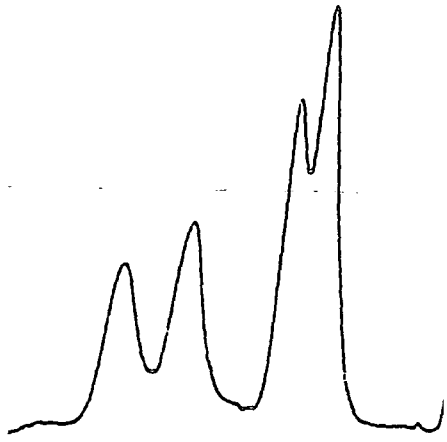

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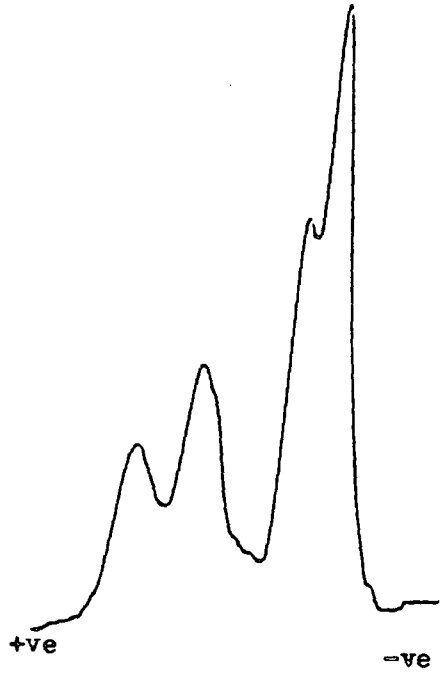

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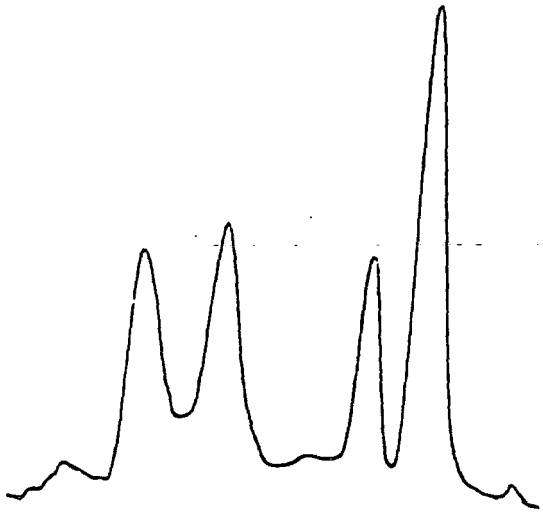

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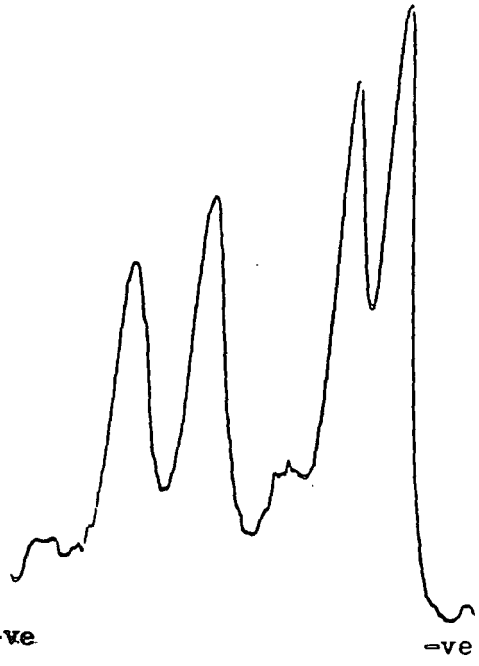

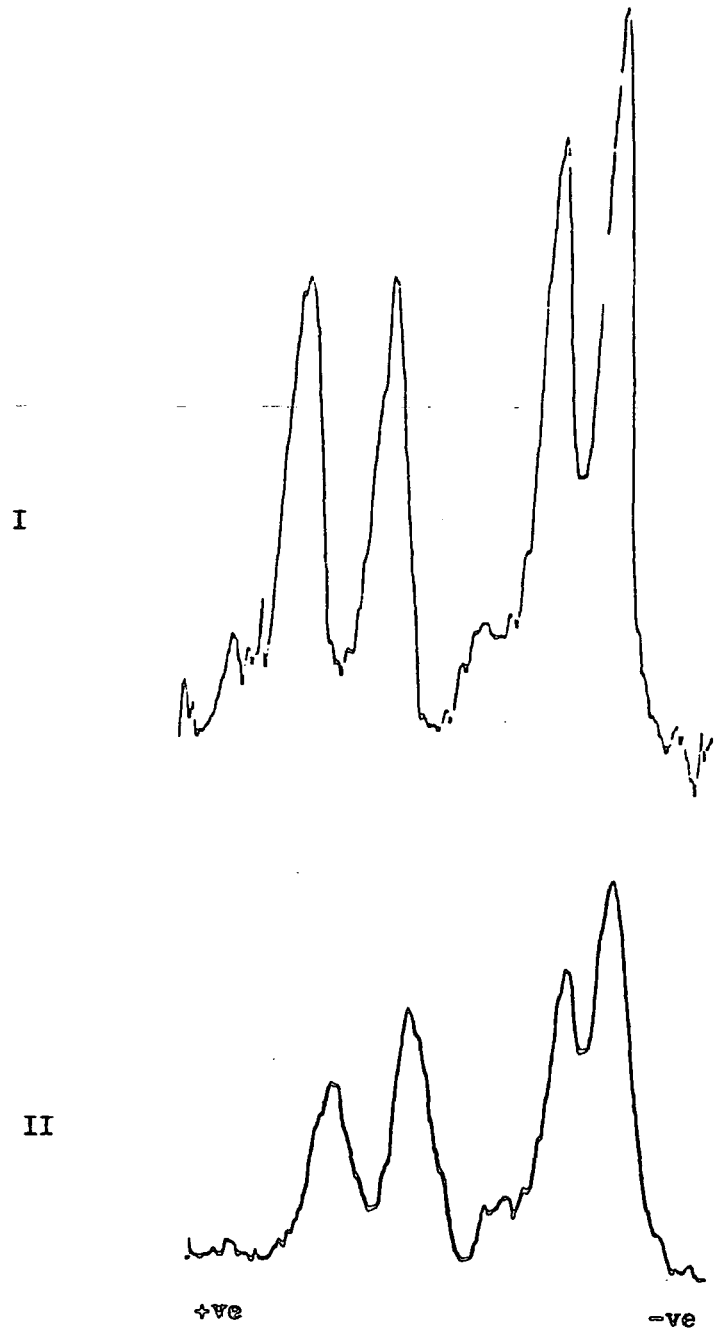
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This may have been due to the tissue reaching the senescence stages.

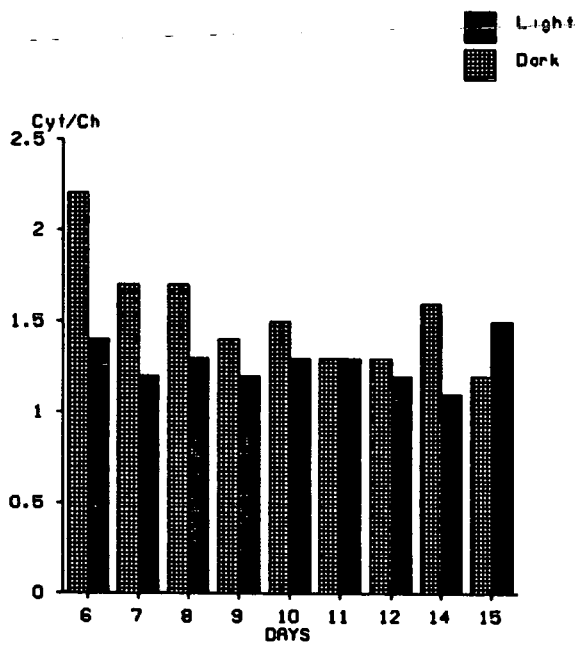
The data in Fig. 4.11 show the ratio between the cytoplasmic and chloroplastic RNA for the tip segments as shown in Fig. 4.10. Fractionation of RNA from leaf tips of seedlings incubated in the light after being grown in the dark, showed a high ratio of cytoplasmic/chloroplastic RNA components, at the early stage of incubation which indicated that the leaf tip may have lost its ability to produce chloroplast components. Alternatively, the cytoplasmic components may have decreased, but this is unlikely during a senescence phase. In contrast, the tip segments for leaves which were incubated in the light, showed a lower ratio of cytoplasmic/chloroplastic RNA components, which indicates that higher levels of chloroplast components were maintained in the light than in the dark.

The data in Fig. 4.12 a–e show the traces of fractionation of cytoplasm and chloroplast components of RNA for the middle segments. A comparison of these results with those for the tip segments (Fig. 4.10) indicated that in the middle segments the chloroplast RNA components appeared to be maintained at a slightly higher level in both the light and the dark.

This is further indicated by a comparsion of the ratios of cytoplasmic to chloroplastic RNA shown for the tip region (Fig. 4.11), and for the middle segments (Fig. 4.13). These differences are probably a reflection of the younger age of the middle segment tissue compared with the older tip tissue.

However, in the light, the ratio between the two components in the middle segments tended to stay the same during sampling period. Again, the level of chloroplast RNA was higher in the light and probably reflects the younger age of the tissue. In the dark-incubated tissues, the chloroplast RNA components were less than those

Figure 4.11



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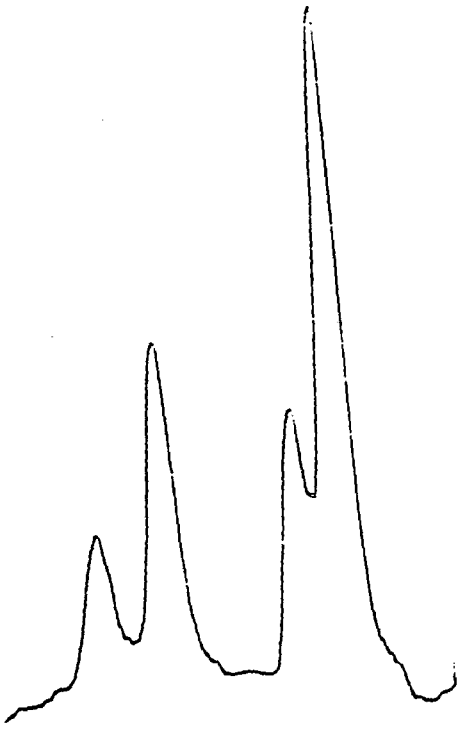

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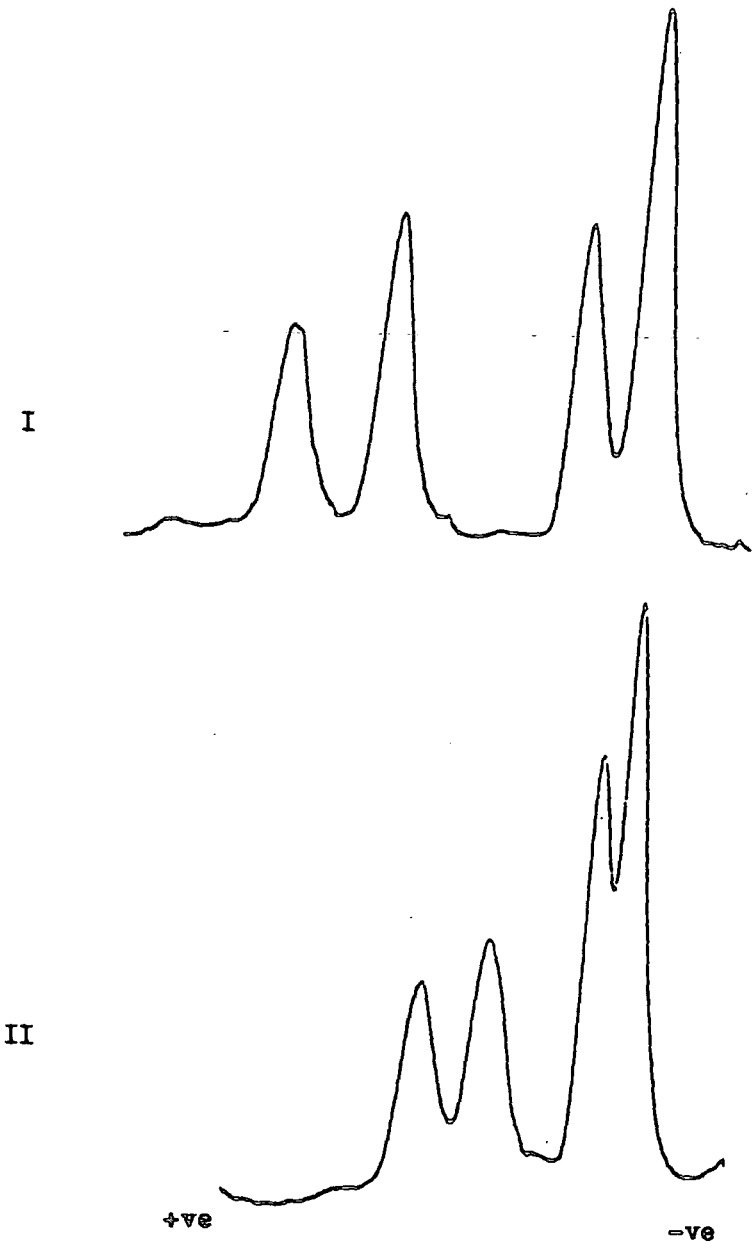
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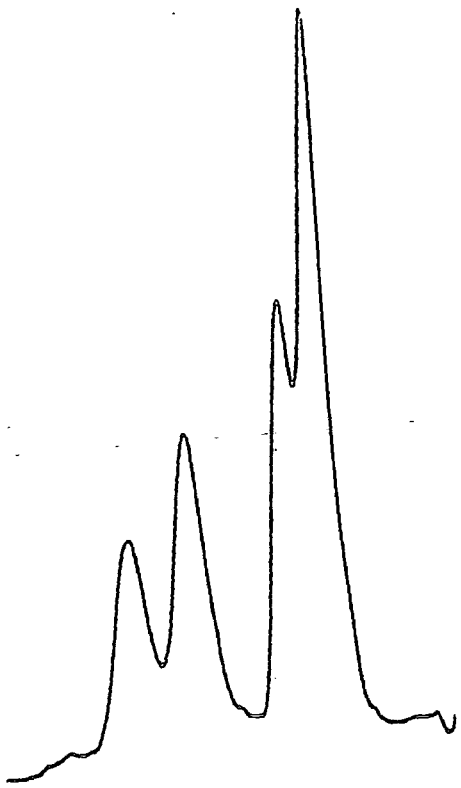

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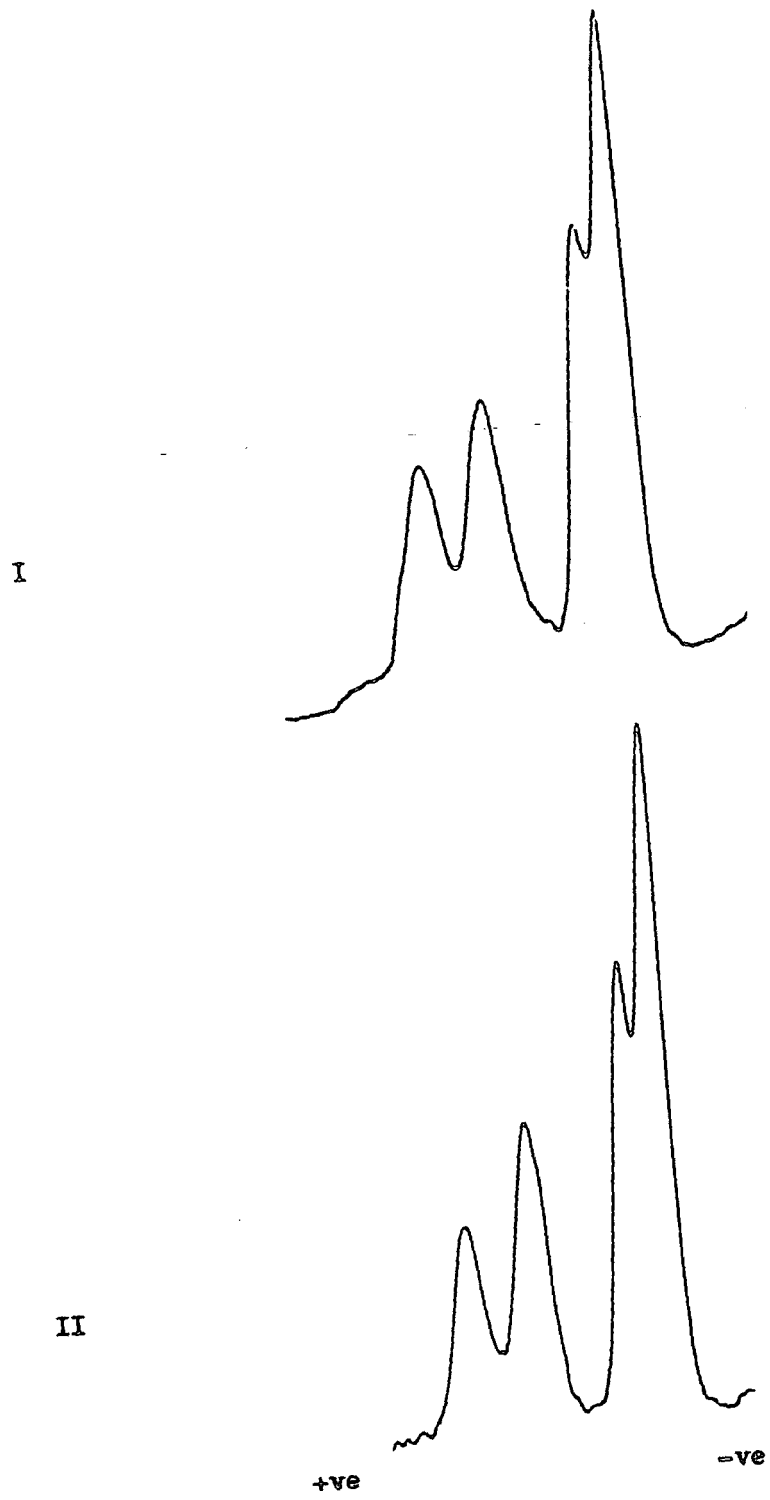
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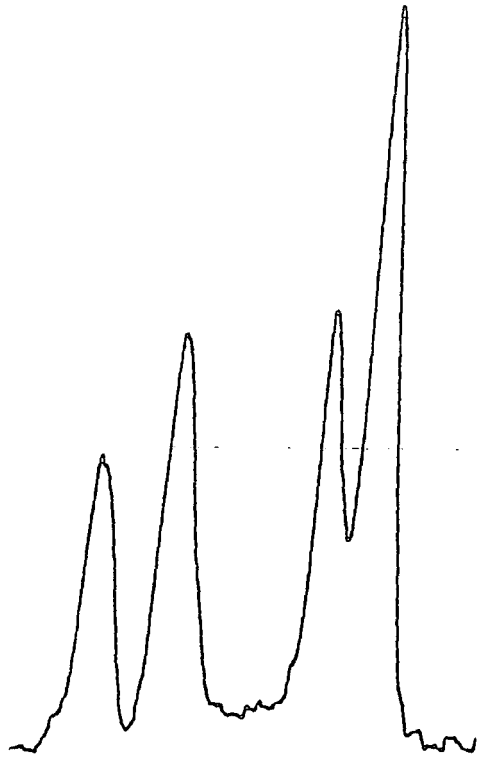

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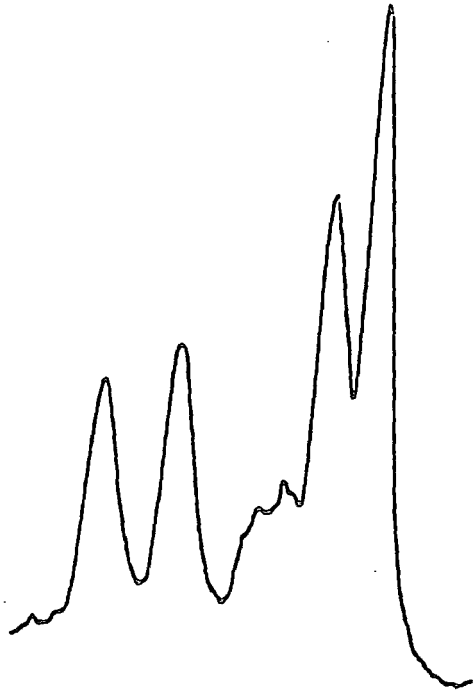

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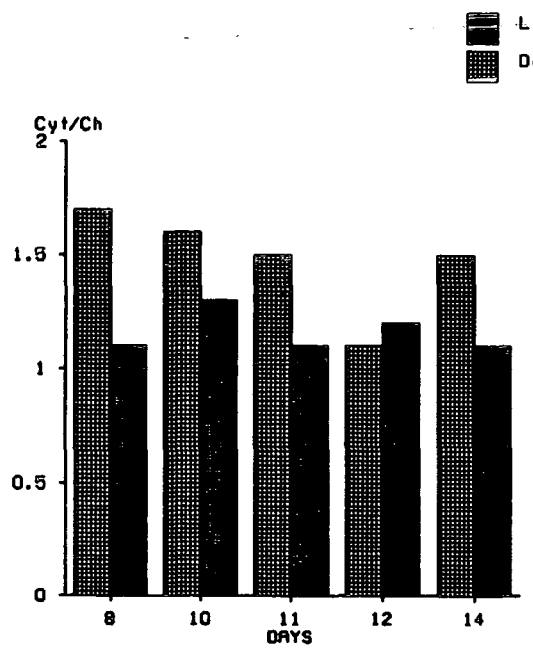
Figure 4.13



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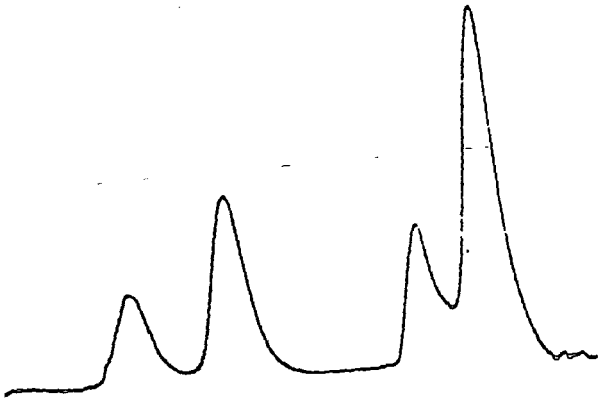

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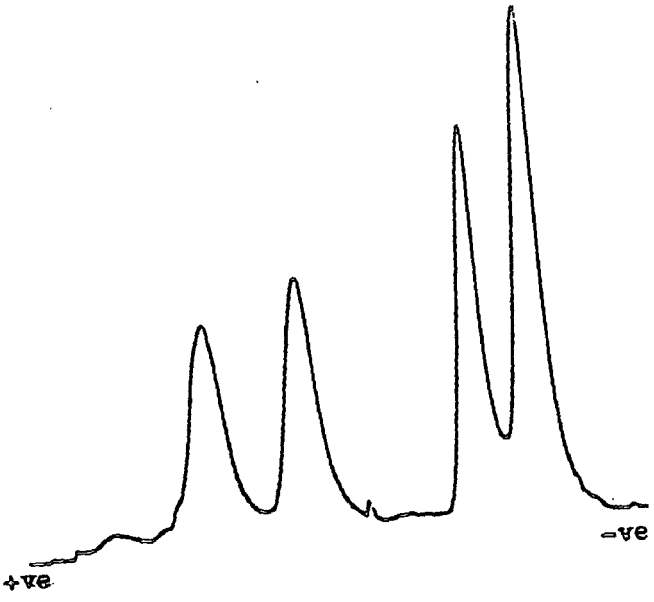

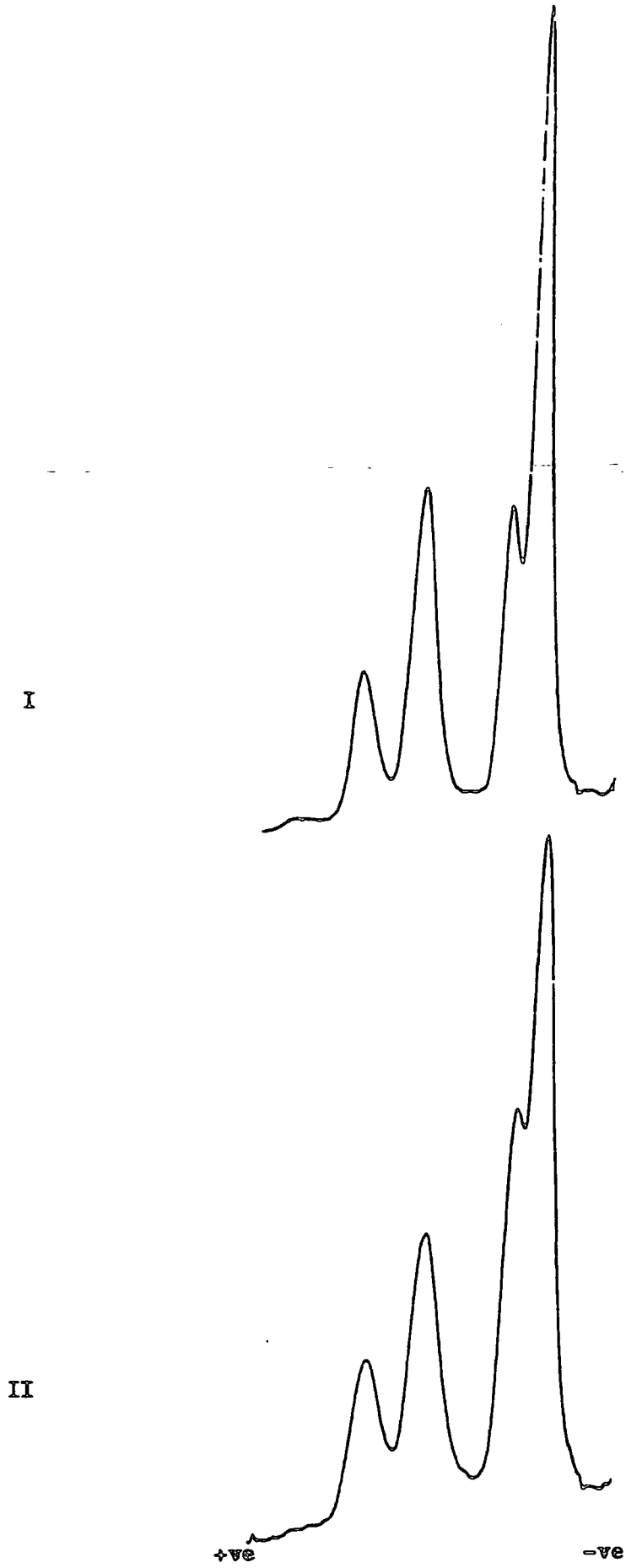
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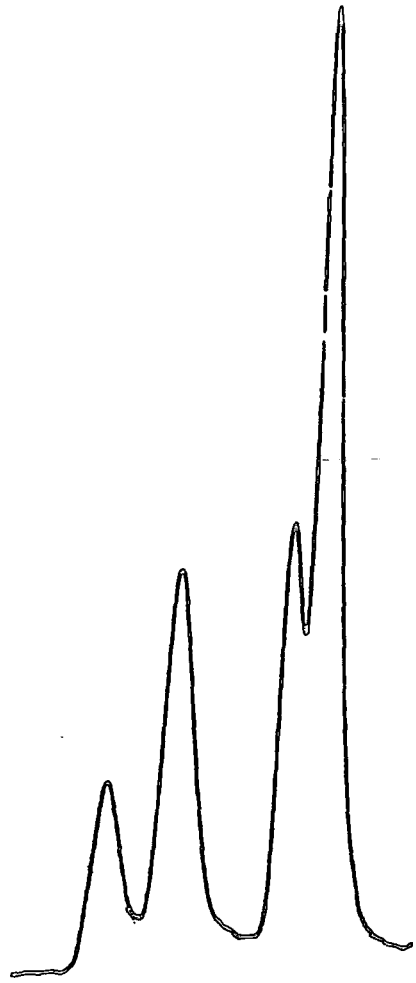

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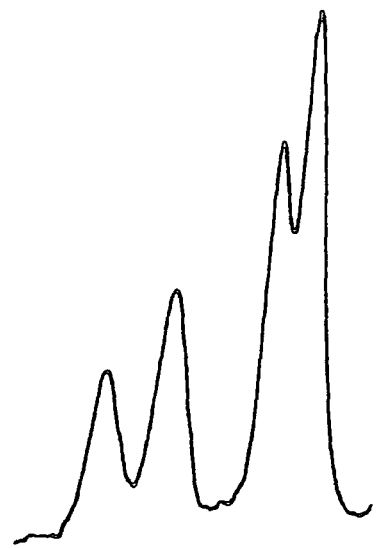

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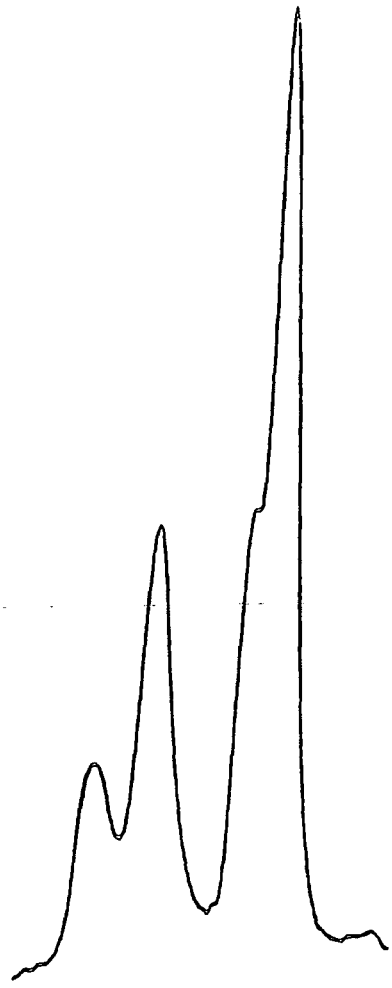

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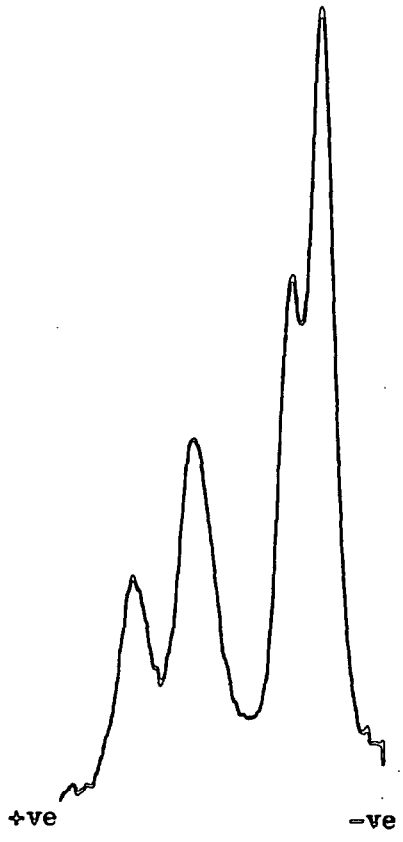

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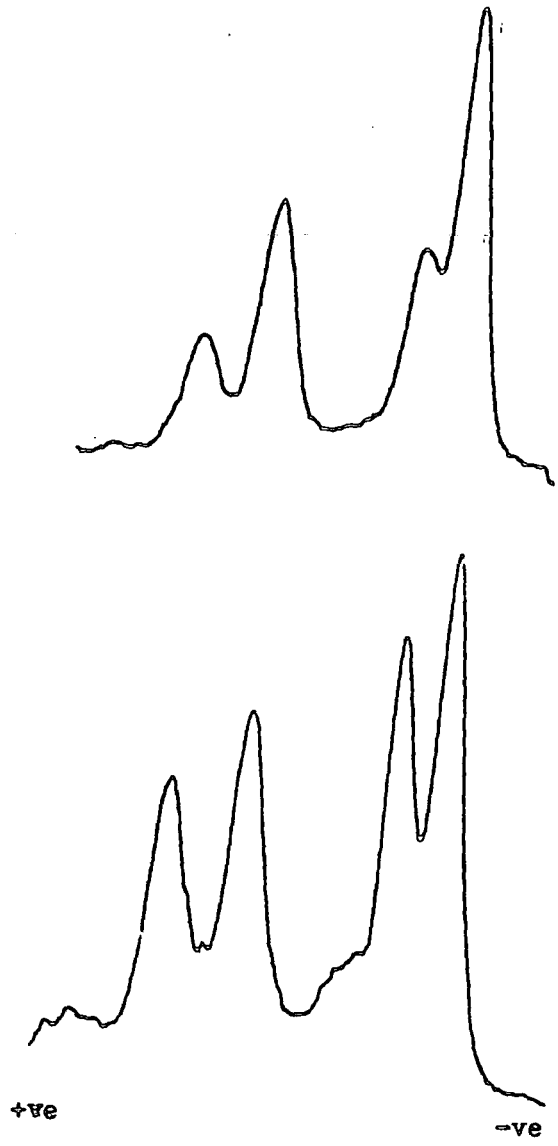

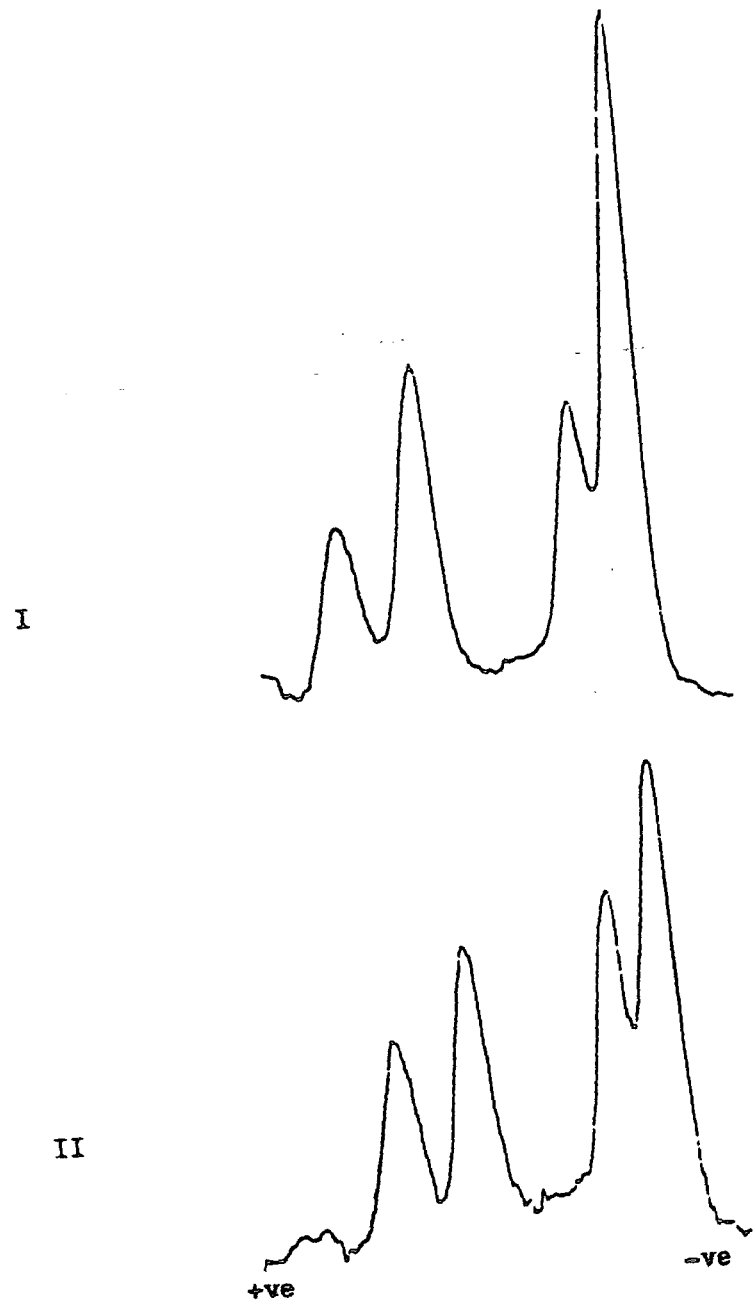
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reported for tip segments.

The data in Fig. 4.14 a–f illustrate the traces of fractionation of ribosomal RNA from basal segments which were incubated in the dark and the light. It was noted that chloroplast RNA components were maintained at a higher level than those in the tip and middle segments.

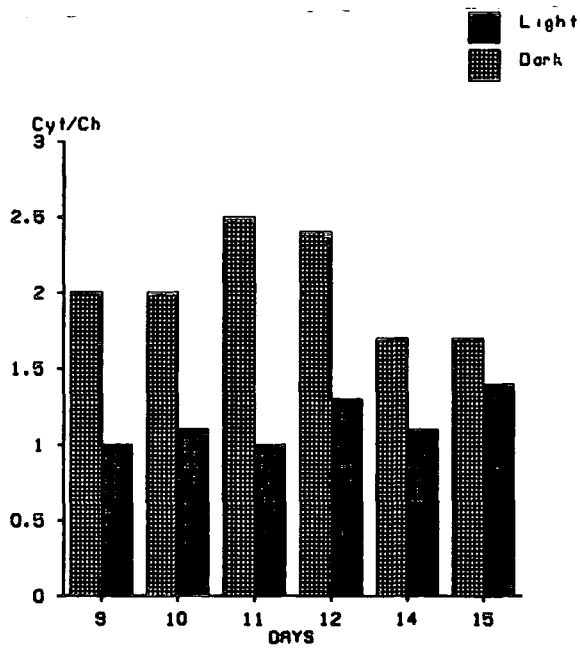
The ratio between cytoplasmic/chloroplastic RNA components for basal segments from day 9 until day 15 is shown in Fig. 4.15. Low levels of chloroplast RNA components were noted in the dark, but these were higher in the light as seen for the other segments. However, after day 15, the tip and basal segments showed slightly lower chloroplast RNA components in the light. At this time it would have been expected that the tissue should have reached the senescence stage but this was not really indicated by the RNA levels.

The data in Fig. 4.16 a–e show the traces for fractionated RNA for tissues which were transferred to the light after 11 days of incubation in the dark. It was noted that chloroplast RNA components were present in the samples fractionated. Therefore, the tissues retained the ability to produce chloroplast RNA components when they were transferred to the light even after this long period of incubation in the dark.

The data in Fig. 4.17 a–c represent the ratio of cytoplasmic/chloroplastic RNA components for the data which were presented in Fig. 4.16. The chloroplast components in the tip and middle segments were at approximately the same level as the cytoplasmic components throughout the period of incubation for 12-17 days, ie cytoplasmic to chloroplastic RNA ratios were approximately 1:1.

In the basal segments the level of chloroplast components was lower than

Figure 4.15



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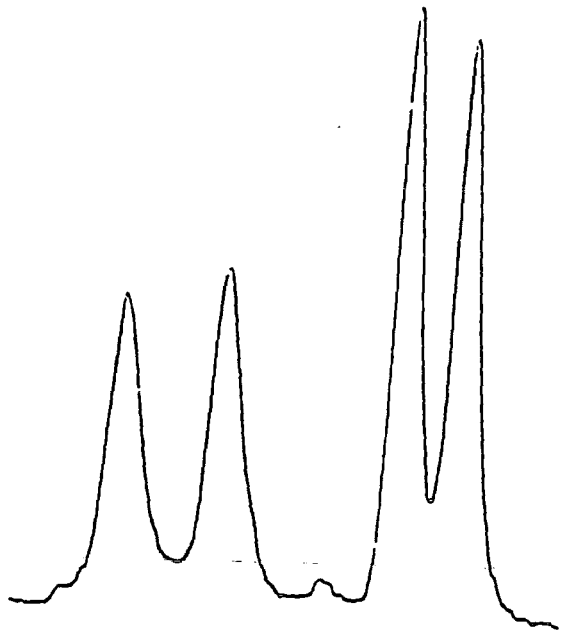

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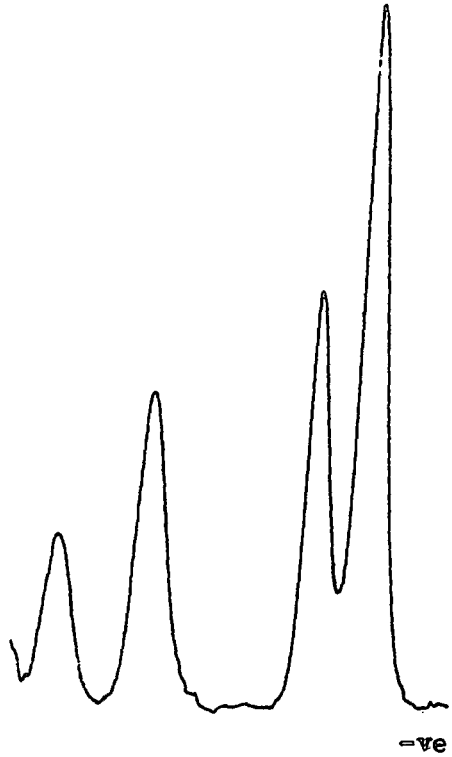

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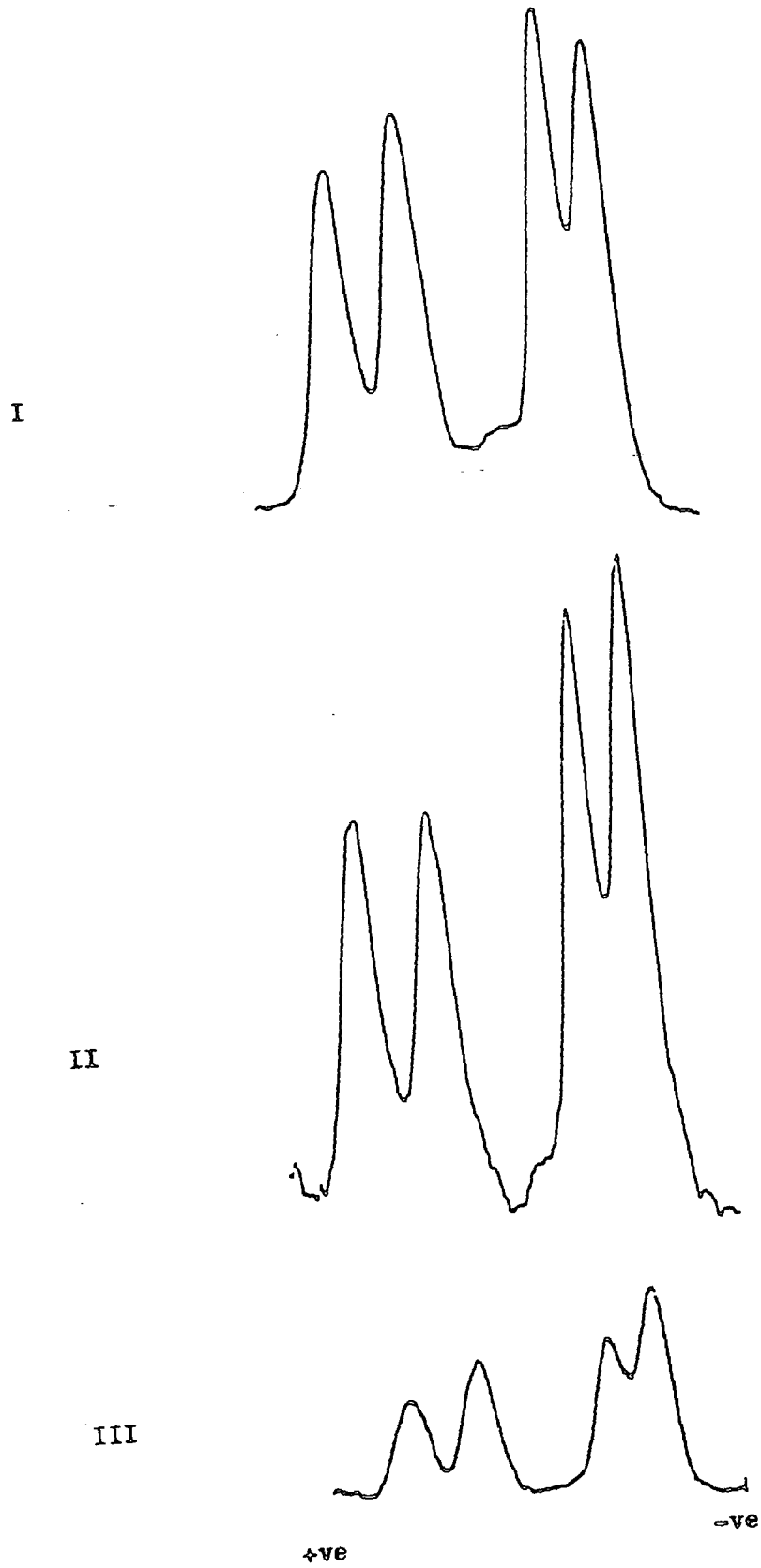
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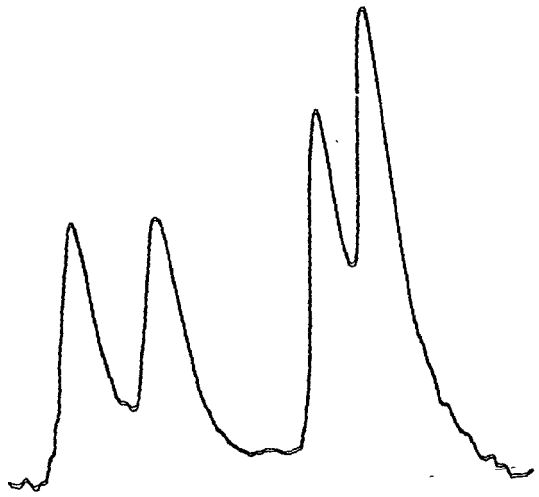

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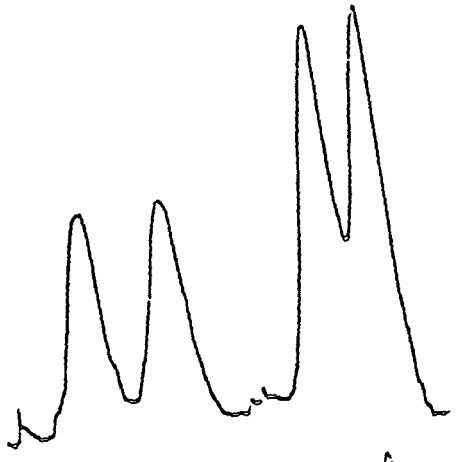

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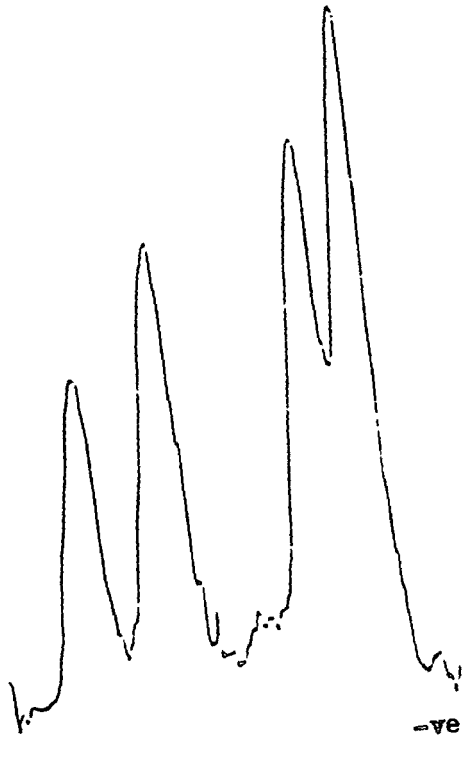

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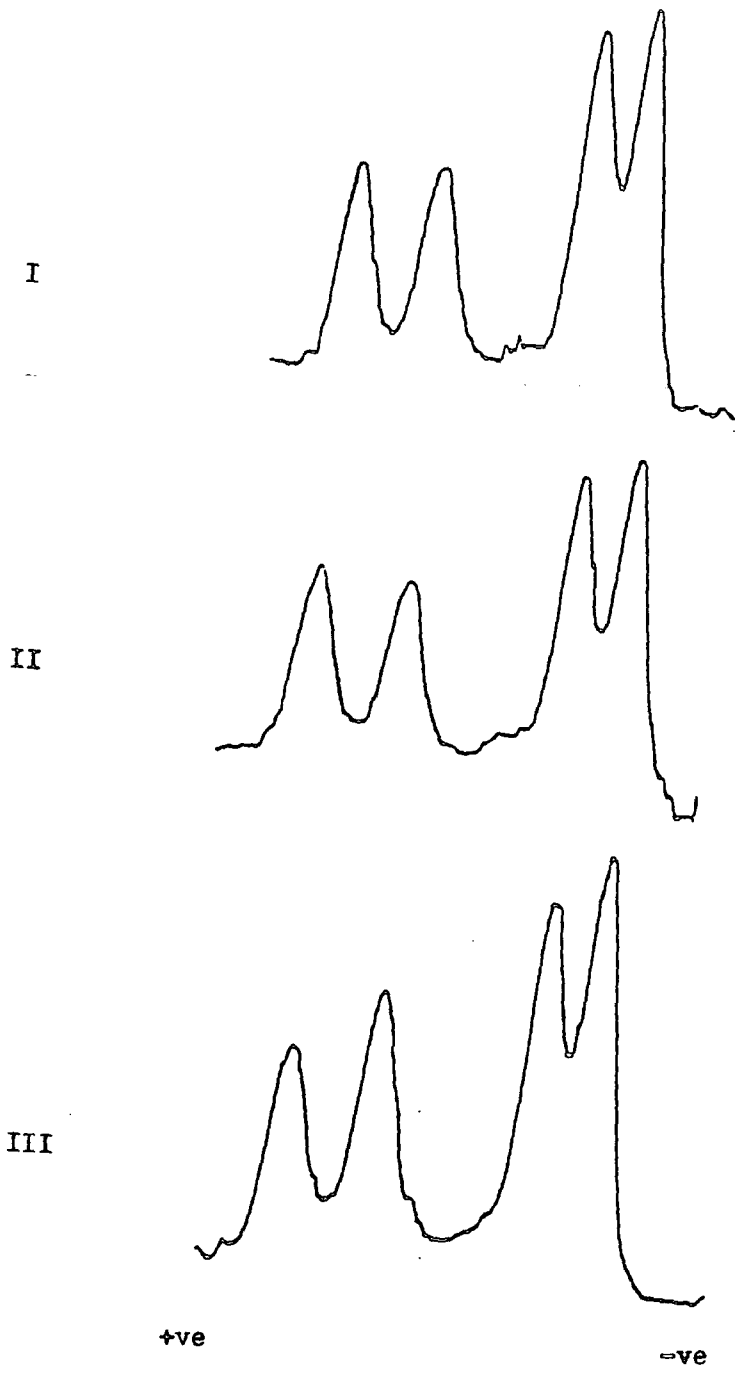
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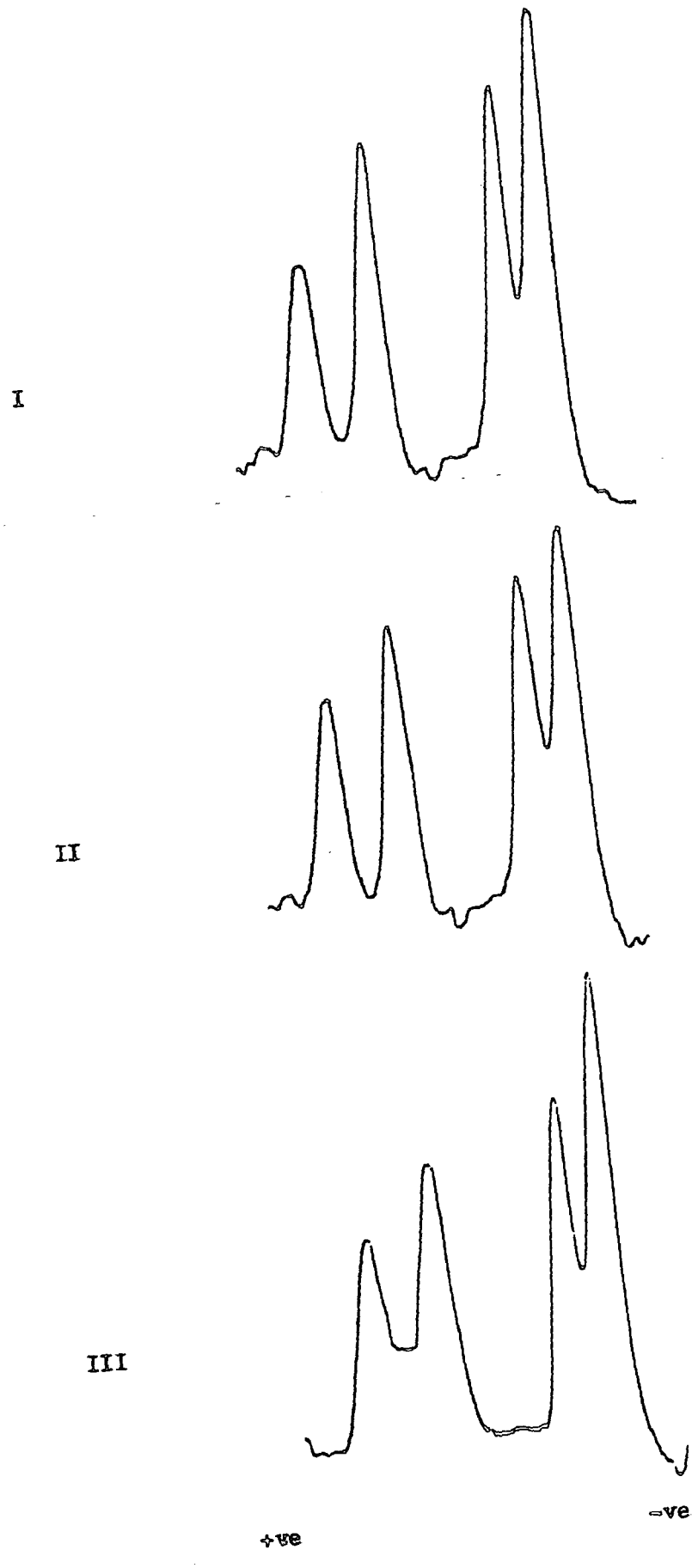
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Figure 4.17

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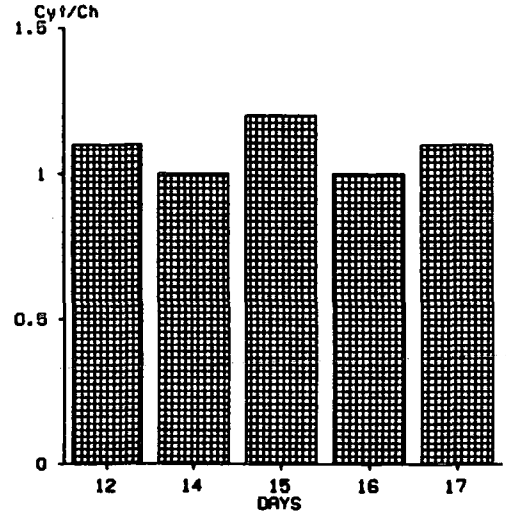

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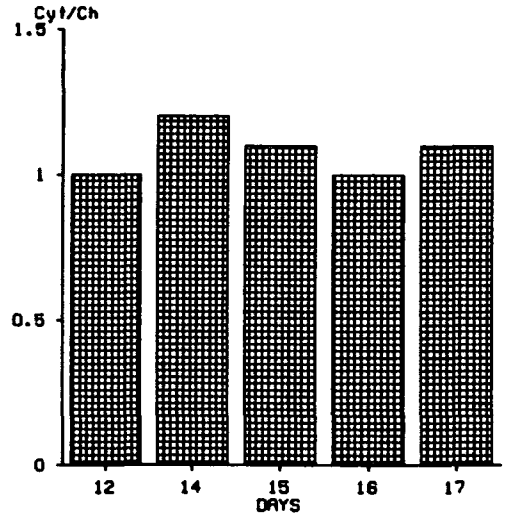

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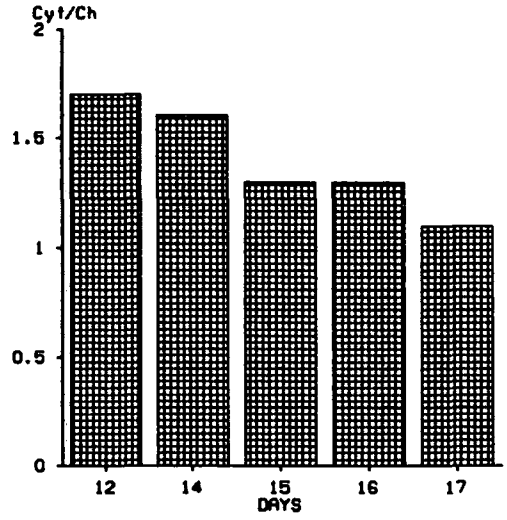

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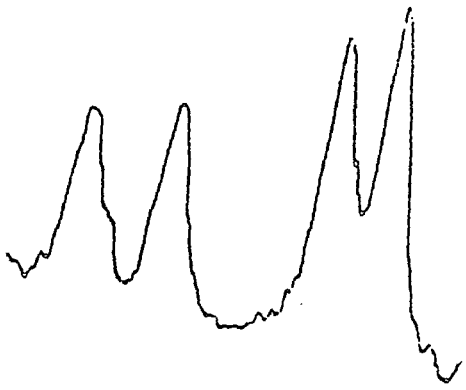

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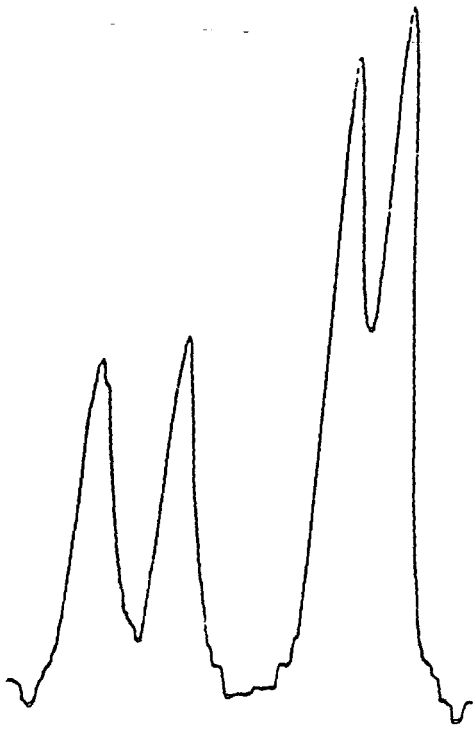

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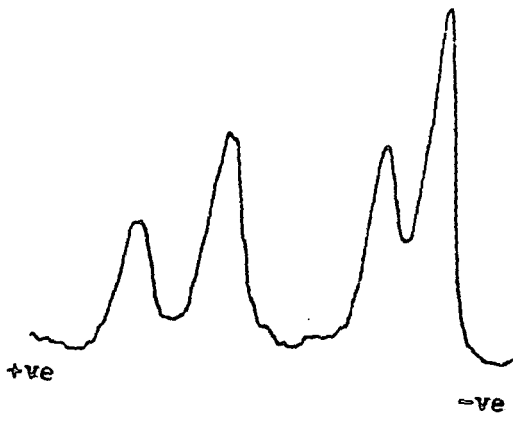

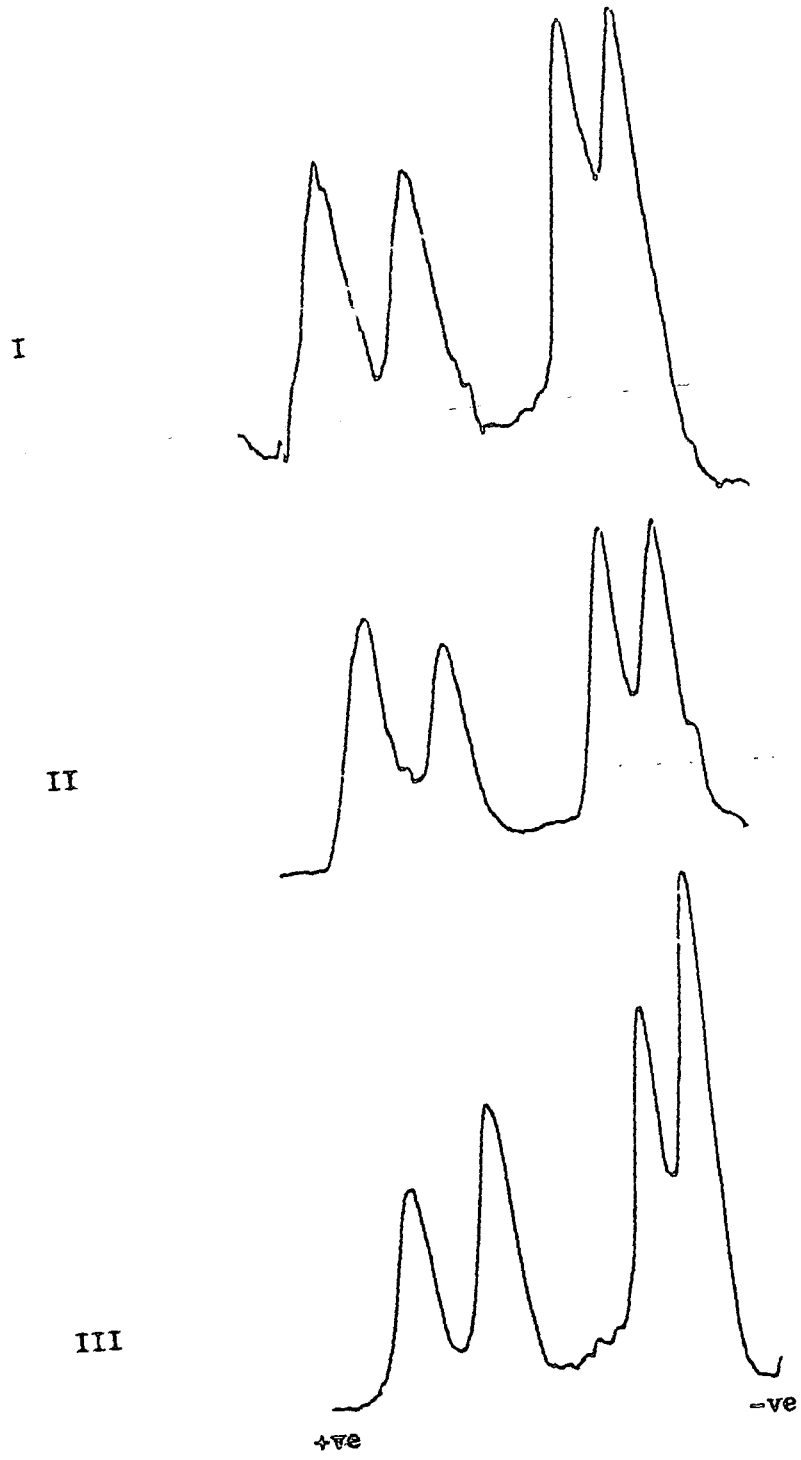
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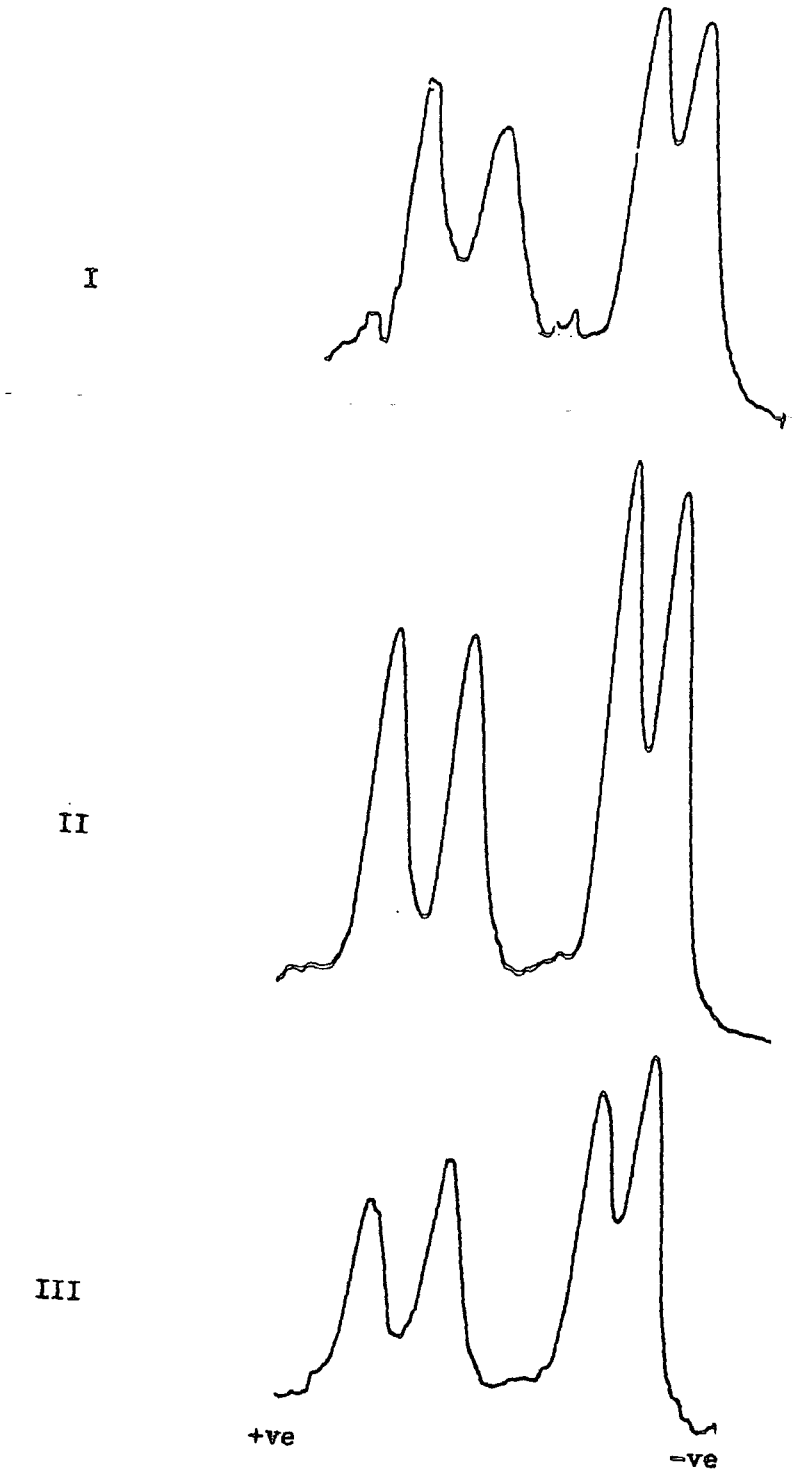
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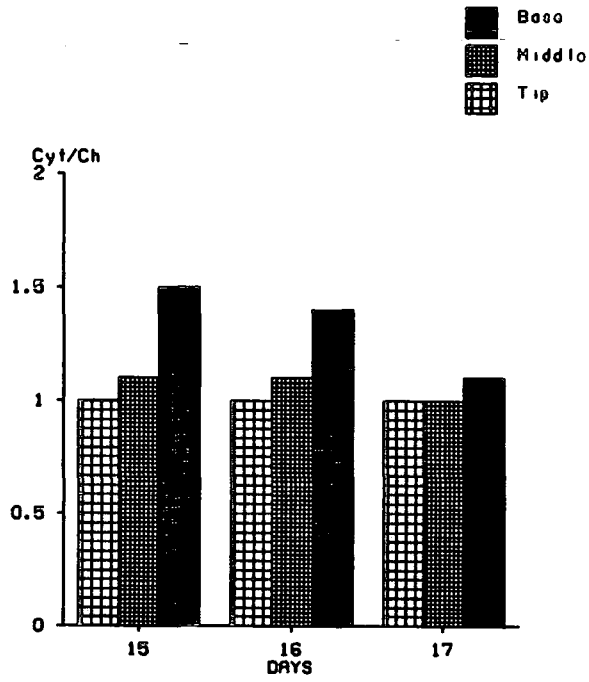
for the other two segments at the begining of incubation, i.e. the ratio of cytoplasmic/chloroplastic was higher. However, over the period of incubation the ratio falls to near unity by day 17. Since the basal segment is composed of the youngest tissue the fall in the ratio represents the continued synthesis of chloroplast components even up to the late stage of incubation at 17 days.

The data in Fig. 4.18 a–c show the traces of fractionated ribosomal RNA from leaves of 14 day-old dark-grown wheat seedlings which were incubated in the light for up to 17 days. The chloroplast components were maintained and the tissue still retained the ability to produce chloroplast components after this long period of incubation in the dark and the light. Given that the tissue had been held in the dark for 14 days prior to illumination for 3 days, it would have been expected that the tissue would have entered a senescence phase. Ordinarily this is indicated by the loss of chloroplast RNA components (Vedal and D'Aoust, 1970), but in this study with wheat, it appears that the chloroplast RNA components are maintained at a high level. This is borne out by the ratios shown in Fig. 4.19.

The ratios indicate that in the tip and middle segments the levels of chloroplastic and cytoplasmic ribosomal RNA are maintained at approximately unity. Again, however, the basal segments show a decline in the ratio indicating continued chloroplast RNA accumulation even at this late stage of incubation. An alternative situation, however, could be that at this late stage of development the cytoplasmic RNA is more labile and is lost in preference to the chloroplastic RNA.

Figure 4.19



4.3.3 Effect of water stress on the NA components of wheat seedlings

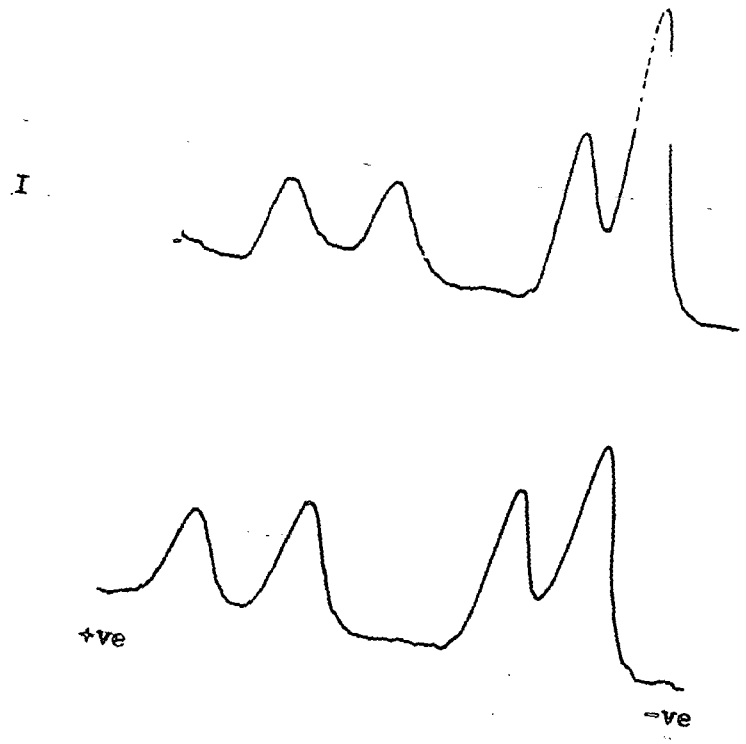
The effects of water stress on total NA of wheat seedlings was variable as mentioned before (page 157). Given that chloroplast RNA components were maintained in the young tissue and old tissue which were incubated for various periods of time in the light and dark the possibility that water stress may have affected these was investigated.

In order to further investigate the effect of water stress on NA components, the effect of treatment of tissue with PEG prior to NA extraction and fractionation was investigated. Wheat seedlings were grown in trays in the dark. They were transferred at day 6 to the light for different periods of time. Seedlings were incubated with their roots in PEG solution or water as described in General Materials and Methods. First leaves were segmented and the RNA extracted from the segments was fractionated as stated before (page 150).

4.3.3.1 Effect of a low concentration of PEG

The data in Fig. 4.20 a and b show the traces of fractionated ribosomal RNA extracted from leaves of wheat seedlings which had been subjected to mild water stress (-5 bar). Chloroplast components were found to be present and maintained in all treatments in both the light and the dark, with or without PEG treatment. The level of chloroplast components was only slightly higher in tissue incubated in the light in water compared with tissue maintained in the dark. PEG treatment appeared to have no effect on the levels of chloroplast RNA components in either the light or the dark.

The data in Fig. 4.21 confirm these results. The ratio between cytoplasmic/chloroplastic RNA components varies only slightly. However, it can be seen that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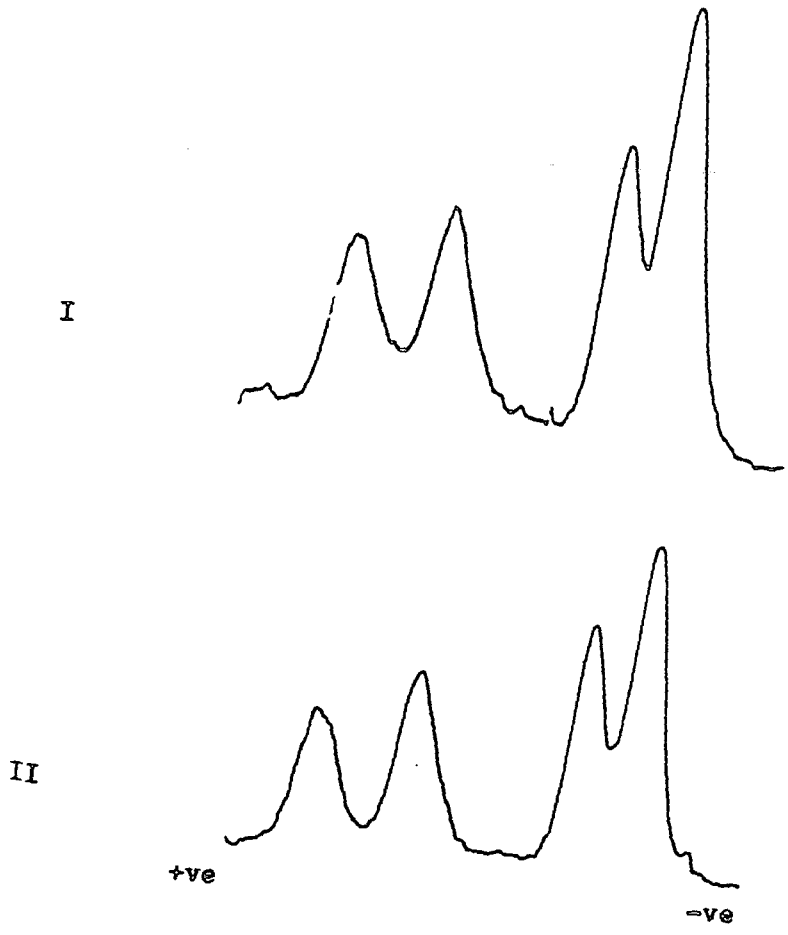
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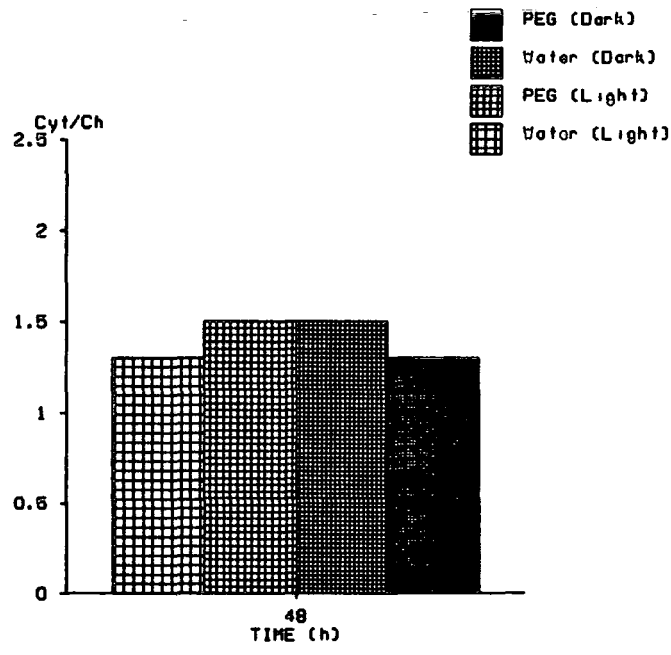
Figure 4.21

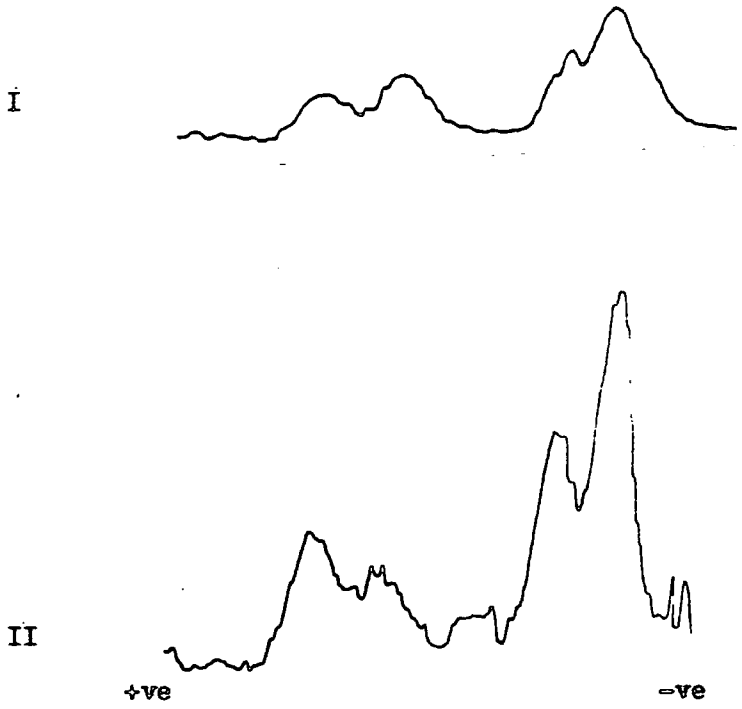

PEG (Dark)
Water (Dark)
PEG (Light)
Water (Light)
Cyt/Ch
2.5
2
1.5
1
0.5
0
48
TIME (h)

the levels of chloroplast RNA components were higher in tissue incubated in the light than in the dark for tissue incubated in water. Also, PEG increased the ratio slightly for light-incubated tissue. However, the levels of chloroplast RNA components were slightly higher in the tissue incubated in the dark than the light, for PEG treatments.

4.2.3.2 Effect of a high concentrations of PEG

The effect of a high concentrations of PEG (-10 bar) on chloroplast RNA components was studied using wheat seedlings treated and incubated as mentioned before (page 214).

The data in Fig. 4.22 a, b and c show the fractionation of RNA components following a treatment with a high concentration of PEG. The PAGE of the RNA components in this fractionation indicated a rapid breakdown in response to treatment. All fractions of RNA seemed to be lost for PEG-treated seedlings,whereas they were maintained for the water controls (Fig. 4.22 d, e and f). Such a loss of RNA could have been due directly to the effect of water stress on the cells or to other cellular changes including the activation of nuclease activity.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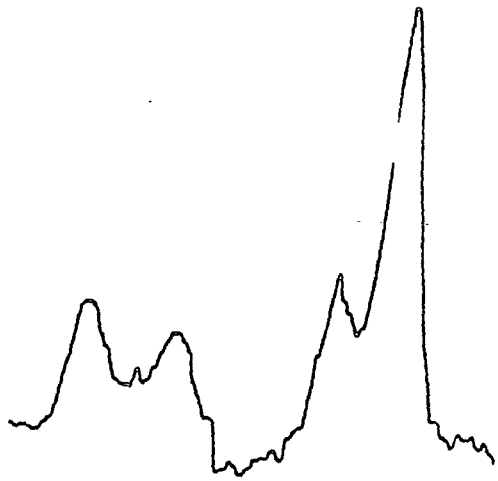

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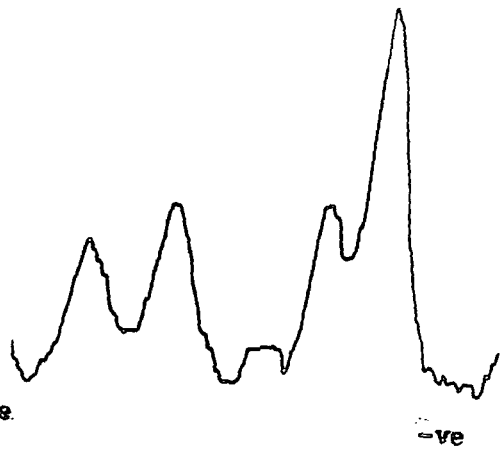

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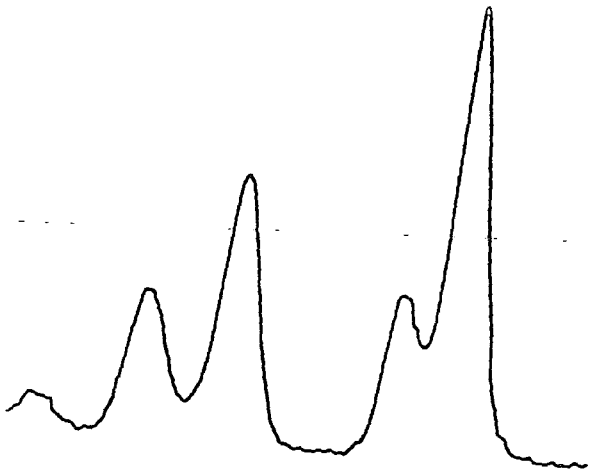

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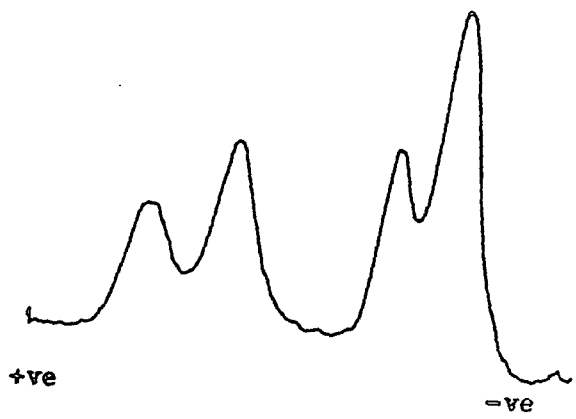

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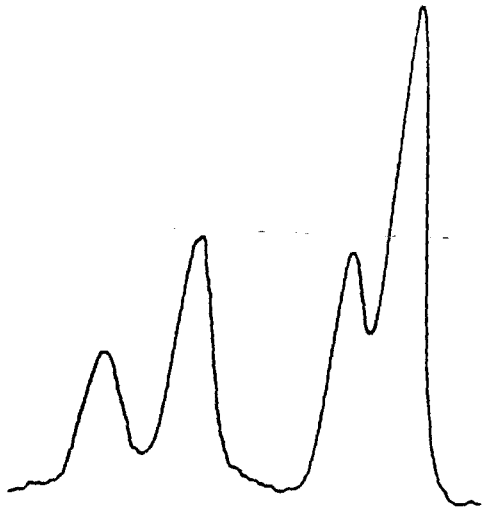

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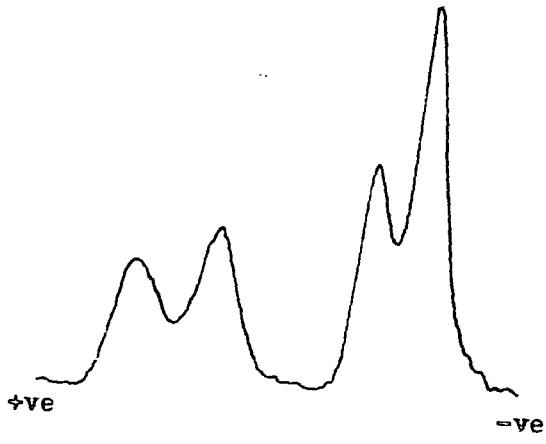

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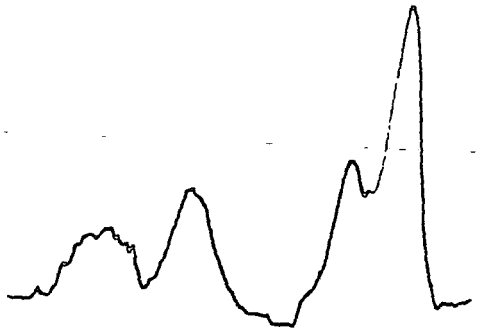

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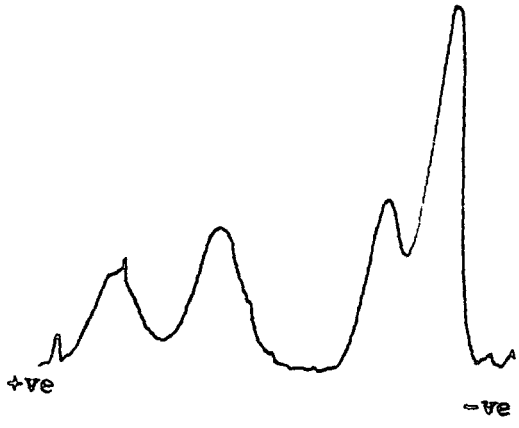

4.4 Discussion

One of the effects of illumination on dark-grown plant organs is accumulation of ribosomal RNA (Wollgiehn and Parthier, 1980; Mikulovich, 1978). The level of NA in dark-grown wheat seedlings was different in their segmented leaves after illumination for 72 h. NA in leaf segments exhibited a different pattern from those of chlorophyll and protein during the greening process. The highest level was accumulated in the middle segments, followed by leaf tips and basal segments.

It has been reported that RNA decreases markedly after excision of leaves in Xanthium pennsylvanicum (Wollgiehn, 1967; Osborne 1962). The factors contributing to decreased RNA levels could be enhanced nuclease activity (Lewington et al., 1967). Dyer and Osborne (1971) found changes in the structure of DNA or a decrease in the synthetic capacity of the leaves due to declining RNA polymerase activity or synthesis of nucleoside triphosphates. Biswal and Mohanty (1976) reported that NA declined in detached barley leaves kept in the darkness. Thimann (1980) reported that loss of NA with time has been recorded for a number of species. Inducing senescence under a variety of artificial conditions appears to result in changes normally associated with ageing and senescence under natural condition.

It has been reported that the increased RNase activity associated with stress conditions, such as water stress (Bagi and Farkas, 1968) and plant bacterial infections (Reddi, 1966), is consistent with the hypothesis that accelerated ageing can be regarded as a consequence of general stress (Farkas and Stahman, 1966). However, in dark-grown tissue, water stress increased the level of NA in young seedlings (6 day-old) in relation to water controls. Wheat seedlings (10 day-old) were less affected by water stress, and the levels of NA was apparently lower. However, the higher concentration of PEG (-10 bar) raised the level of NA in young seedlings after 72 h in

the light and dark. Furthermore, similar responses were found with dark incubated wheat seedlings. However, water stress had no effect on the NA of roots of the same seedlings. It has been shown previously that there was a large increase in total NA during osmotic pre-treatment of tomato seed (Coolbear and Grierson, 1979).

Therefore, the effect of water stress had transferred via the root to the shoot systems. However, there was no effect of water stress on the level of NA of excised leaves or leaf segments. This finding is similar to that reported with chlorophyll (Chapter 2) and protein (Chapter 3), which indicated that water stress had no effect on the cellular components of excised leaves or leaf segments.

The results of the effect of water stress on the NA fractionation wheat seedlings in this study are in contrast with the finding by Todd and Basler (1965) working with different varieties of wheat exposed to various levels of water stress. They found a decrease in NA content in various sub-cellular fractions of leaves and crown and suggested that death of the plants occurred whenever the NA content of any given fractions dropped below a certain critical level. Similarly, Rhodes and Matsuda (1976) reported that a decrease in polyribosomes in pumpkin seeds was detectable following their exposure to an osmotic stress. Similar results were reported by Cocucci et al. (1976) with shoot tissue of peas, barley and wheat. The decrease in polyribosomes has also been seen in water stress inhibited growth of squash fruit. Armstrong and Jones (1973) found that osmotic dehydration in - 6 M PEG reduced the binding of ribosomes to the endoplasmic reticulum in barley aleurone cells.

In this study K treatment resulted in an increase in the levels of NA in young seedlings after a period of 48 h in the light. However, there was no further increase after 72 h. K treatment did not appear to affect the level of NA in old seedlings. In the dark treatments K slightly raised the level of NA after 72 h.

In contrast, K treatments on the roots of wheat seedlings had no effect on the level of NA. Similar patterns of response were found with excised leaves and leaf segments, when the tissues were incubated with K, and there was no effect in the level of NA in the tissues. However, it has been reported that in senescing barley leaves, the activity of RNA polymerase was higher in cytokinin-treated material than in the untreated material (Schneider et al., 1978; Schneider, 1980). It was found that the presence of cytokinin in tRNA's increased the ribosome binding affinity for the aminoacylated tRNA. (Fittler and Hall, 1966). Berridge et al. (1970) showed a binding of the cytokinin to the 80S ribosomes, with a correlation between the extent of binding and the biological activity of various cytokinins and cytokinin analogues. Mikulovich et al., (1978) reported that cytokinin stimulated the incorporation of radiolabelled components into RNA in isolated chloroplasts through activation of RNA polymerase systems of chloroplasts.

In this study with wheat, however, the level of NA was increased with a combination of K and PEG in the young seedlings after 48 h, in relation to K treatment alone. Similar responses were found in the light and dark treatments. There was no effect of the same treatment on the level of NA in the roots of the wheat seedlings. The results were variable and may reflect tissue age and period of incubation. Moreover, in excised leaves and leaf segments a combination of K and PEG did not bring about an increase in the level of NA.

During experiments using leaf segments of wheat seedlings for periods up to 17 days in the dark and light starting from day 6, the level of NA declined in all leaf segments, and was higher in the dark than in the light. However, after a long period in the dark (11 days), and 6 days in the light there was general decline in the levels of NA by the end of incubation periods. Furthermore, middle segments represented

the highest level of NA after the period of incubation, followed by the tip and least in the basal segments. Again, after further incubation in the dark (14 days), the level of NA was higher in the basal segments, followed by middle segments and least in tip segments. These variations in the level of NA of segmented leaves may be due to a difference in metabolism and physiological conditions between leaves. This could be related to the physiological age of the tissue where the concentration of various compounds would vary. It is possible that such a variation could involve a decreased level of endogenous cytokinin in the older tissue which could result in reduced RNA levels.

Fractionation of NA from segmented leaves of incubated wheat seedlings. by PAGE gave some insight into the qualitative changes in NA underlying the quantitative changes. All extractions gave more or less identical results for the electrophoresis pattern of the RNA components in that cytoplasmic and chloroplastic heavy ribosomal components were present. However, the relative proportions of the components varied with the developmental stage of the tissue. The older tissue contained a lower proportion of chloroplast components. However, the fractionation of the segments of dark grown leaves showed that the components of cytoplasmic and chloroplastic RNA were maintained in all segments (tip, middle and base) even after extended periods of incubation. Furthermore, in a comparison between components in the light and dark, it was clear that chloroplast RNA components were higher in the light than the dark as could be expected.

Fractionation of leaf segments grown from day 6 to day 15 in the dark and light, showed that chloroplastic and cytoplasmic RNA was maintained during the period of incubation in the dark and the light for leaf tip segments. Similar responses were found in the middle and basal segments. It was noted that the cytoplasmic

RNA was higher in samples incubated in the light compared to those incubated in the dark. Since ageing of leaf tissue has been shown to result in an early decline in heavy ribosomal RNA fractions, it was expected that similar effects would have been found in this study. Vedal and D'Aoust (1970) found that there was a 57% breakdown of the 23S RNA in etiolated radish cotyledons by day 16 compared to only 29% of day 6. In green cotyledons, 23S chloroplastic RNA degradation increased with age and 23S RNA breakdown was greater in dark than in green tissue. The fact that ribosomal RNA fractions were maintained in wheat tissue indicates that these fractions do not change in this particular species as early as in others. Alternatively, it is quite likely that the extraction methods used in this study (presence of DEP and SDS, both nuclease inhibitors) indicated a greater preservation of the RNA components.

It is, however, difficult also to make direct comparisons between individual species since the patterns observed in monocotyledenous leaves will differ from those in dicotyledenous leaves. In the former there is a defined age sequence down the leaf whereas this is not always the case for the latter where the whole leaf is effectively of the same age.

Comparison of the levels of specific fractions indicated that the basal segments contained the most chloroplastic RNA components followed by middle segments and these were least in the leaf tip segments. However, there was a loss of definition for all RNA components after a long period of incubation (17 days), which may be due to leaf tissue reaching the senescence phase with a subsequent breakdown of RNA due to nuclease activity.

The ratio between cytoplasmic/chloroplastic RNA components gave a similar pattern as the traces of fractionation of ribosomal RNA for all segments in this respect. Long periods of incubation in the dark did not stop the tissue maintaining ribosomal

RNA components in the light. After a long period of incubation in the dark (11 and 14 days), all leaf segments showed a similar pattern of fractionations when the leaves were kept in the light (up to 17 days). The ratio between cytoplasmic/chloroplastic for those kept in the dark for 11 and 14 days varied along the leaf. The leaf tip segments showed the highest chloroplastic RNA components followed by middle segments and the least in basal segments.

In studies with pea leaf tissue (Pisum sativum) Mills and Baumgartner (1983) reported that chloroplasts from 7 to 9 day-old plants contained higher numbers of plastids compared to those from 14 day-old plants. Therefore, as far as species could be compared, it would expected that loss of chloroplast components should have been apparent in the older wheat tissue, but this was not readily seen for the chloroplast RNA components.

It has been reported that in dark-grown cotyledons, the relative increase in plastid rRNA contents subsequently decreased (Thien and Schopter, 1975). Ingle (1968) and Vedal and D'Aoust (1970) reported that in radish cotyledons the accumulation of chloroplast RNA was stimulated by light. Vedal and D'Aoust (1970) reported that light was not an absolute requirement for proplastid RNA synthesis, a slight accumulation being observed in the dark. Bogorad (1976) and Harel and Bogorad (1973) reported that in greening maize leaves, the light-stimulated chloroplast rRNA synthesis. In isolated cotyledons, light caused an increase in the amount of both chloroplast and cytoplasmic ribosomal RNA and was higher for plastid rRNA (Roussaux et al., 1976; Mikulovich, 1978).

The influence of water stress on the maintainance of the ribosomal RNA components showed contrasts between the levels of treatment given. Whilst exposure of the tissues to -10 and -5 bar PEG treatments resulted in only very marginal changes

in the total NA levels after 3 days of treatment in the light following growth for 6 days in the dark changes were seen in specific fractions. Mild water stress treatment (-5 bar PEG) for up to 48 h had no apparent marked effect on the specific ribosomal fractions when compared with water controls. This is consistent with observations that this treatment did not alter markedly the quantitative yields of RNA. Similar, results were found in both the light and dark incubated tissue.

Tissue incubated in the higher PEG concentration showed a degradation of both chloroplastic and cytoplasmic ribosomal RNA components in both the light and the dark even after 24 h when compared with water controls. At 72 h of PEG treatment there was a marked loss of RNA integrity. The fact that the light treatment gave essentially the same results as incubation in the dark indicates that chloroplast function and integrity were being impaired as well as there being effects on the cytoplasmic ribosomes.

Kessler (1961) found that water stress appeared to impair NA accumulation; however, here the quantitative yields of RNA were not markedly altered by the higher PEG treatment. Kessler (1961) similarly reported that the breakdown of plastid ribosomes which preceded the disappearance of cytoplasmic ribosomes. Mittelheuser and Van Steveninck (1971a) reported a similar sequence in disappearance of ribosomes under severe water stress conditions. The results here indicate that for wheat the loss of chloroplastic and cytoplasmic ribosomes (measured in tissues of their RNA stablities) was more or less equal.

CHAPTER FIVE

5. General Discussion

The preceding chapters have given the results of an investigation of greening and development of wheat seedlings in relation to the age of the tissue, time of pre-incubation in the dark and imposed water stress. Discussion in previous chapters has already emphasized the changes in the levels of cellular components during the greening process and development and the effect of water stress on these processes. In this study the pattern of change of all cellular components in wheat seedlings, excised leaves and leaf segments was monitored during leaf greening and development of the experimental systems used. The intact plant offered a better environment for the study of leaf development than either the excised leaves and leaf segments.

There were variations in the pattern of chlorophyll, protein and nucleic acids under the various treatments in intact plants during greening and development. In contrast, excised leaves and leaf segments showed relatively small changes in the cellular components during greening other than in the levels of chlorophyll. This is not surprising in view of the knowledge that each region of an intact plant has the benefit of materials made by the rest of the plant as it greens. In contrast, the excised leaves and leaf segments have only the resources and developmental capacity of their own cells. The changes in the levels of chlorophyll during greening reported by other workers using intact tissue were probably due to differences in plants species or experimental methods. Conditions under which plants were grown and their subsequent handling could also be contribute to observe a differences in chlorophyll levels.

As early as 1920, Briggs found that the age of tissue affected the rate at which it developed the ability to liberate and incorporate CO_2. Also Obendorf and Huffaker

(1970) showed that the age of dark-grown barley seedlings had a significant influence on light-induced changes in chlorophyll synthesis, protein content and activities of several Calvin cycle enzymes.

In this study with wheat, it was shown that ageing of tissue in the dark prior to illumination reduced the capacity for chlorophyll accumulation. This effect was seen within the leaf, ie the tip being the oldest portion, and between leaves of different ages. Even though it was not measured, CO_2 exchange and therefore photosynthetic capacity could be expected to have been reduced in the aged tissue. Also, the changes in the capacity to accumulate protein and nucleic acid components would influence the formation and functioning of the chloroplast.

The content of cellular components during greening of dark-grown wheat seedlings were sensitive to water stress. The rapid decrease in leaf water potentials between treatment with PEG at -5 bar and -10 bar coincided with the end of cellular component accumulation. These observations clearly demonstrate that cellular component accumulation is influenced primarily by environmental conditions prevailing during development and greening of dark-grown tissue and is not indicative of programmed events in leaf development.

The leaves of the wheat seedlings under water stress wilted, but they did not dry completely even though the leaf tips were desiccated. Whilst the use of PEG was an artificial means of water stressing the plants, this compound, and related compounds have been reported as being active as in natural conditions of water stress. Hodgson et al. (1949) showed that polyethylene glycols, polyvinyl alcohols and polysaccharides from crown gall bacteria caused wilting in mature leaflets at low concentrations. Lawlor, (1970) summarized the mechanisms of PEG during water stress:-

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

2- PEG interfered with plant metabolism

3- PEG lowered the surface tension within the leaf, affecting cell permeability

4- PEG blocked the transpiration pathway.

The effect of water stress was also noted in the roots of the treated seedlings. Here growth was inhibited as was the pattern of protein and nucleic acid accumulation. This effect was similar to that seen in the leaves. The effect, therefore, on the leaves could have been in part the result of the effect on the roots. This could also be consistent with observation that water stress effects were not as severe on excised leaf tissue.

Roots under water stress were yellow with the intensity of the yellow colour related to the amount of stress applied. This was clearly apparent between the stress and non-stressed roots of the same plant. Zgurouskaya and Tsel'Niker, (1955) reported similar results with roots under soil moisture stress. The colour may be due to the formation of a particular pigment under stress (Zgurouskaya and Tsel'Niker, 1955) or an accumulation of pigments in general (Nezgovorova, 1957) or a change in state of various compounds.

Given the potential limitations to the use of PEG, the studies reported here with wheat indicate that water stress conditions imposed by the use of this compound did cause alterations in cellular component accumulation pattern in the plants. The levels of chlorophyll accumulated in illuminated tissue which was previously dark-grown were subject to variation with PEG treatment. This was the most consistent effect and could be expected. Protein level changes, however, were more complex. In

addition to the effect of water stress in potentially involving accumulation of protein associated with formation of the photosynthetic apparatus, there was the potential effect of altered protein levels with these components acting as stress metabolites. The more severe water stress (-10 bar) treatment had the more marked effect on nucleic acid components. It was noted that both the cytoplasmic ribosomal components were lost as were as those from the chloroplast. This indicates a compounding effect of water stress on the central metabolism of the plant other than on the chloroplast alone. This is in contrast to the results of Brady et al. (1979) who found that cytoplasmic ribosomes were lost in preference to those from chloroplasts.

There appeared to be a correlation between the pattern of cellular components accumulated and the growth inhibition due to water stress. The least effect on growth and the components was seen with -5 bar PEG, whereas -10 bar PEG had a greater effect. It is not possible to conclude from this study if growth was inhibited indirectly by an effect of water stress on photosynthesis as noted by Bhardwaj and Singhal (1981) for barley or through a direct effect as the cell growth pattern.

The involvement of phytohormones in the adaptive responses of plants to water stress has been reported. Cytokinin levels in xylem exudates were reported to be depressed by water stress (Itai and Vaadia, 1971) and the effects of cytokinins on stomatal apertures and plant growth were opposite to those of ABA (Mizrahi et al., 1970; Khan, 1969). A reduced cytokinin content would tend to augment the effect of higher ABA levels on stomatal closure and growth. The use of K in the study reported here was to investigate if its addition to the plants could lessen the effects of imposed water stress on cell component accumulation. At the same time this compound could counter any effects of ABA which may have accumulated in the tissue. It has been reported that during water stress a rapid formation of ABA occurs. The levels of

this compound increased by up to 40-fold in green wheat leaves subjected to severe water stress (Wright, 1969; Wright and Hiron, 1969). The chloroplast has been demonstrated to be the site of ABA synthesis in the leaf (Milborrow, 1974; Railton et al., 1974; Loveys, 1977). Furthermore, whilst ABA is synthesized in the chloroplasts it might be released for a regulatory role in the cytoplasm (Willburn et al., 1973; Willburn and Hampp, 1976). Raschke (1975) suggested that ABA may extend its effect on an H^+ expulsion mechanism in the plasmalemma. Cleland (1977) found that increased cell wall extensibility and cell growth occurred, when cells were caused to excrete hydrogen ions, lowering the pH of the cell wall solutions. It seems possible that in the water-stressed plant, ABA inhibited H^+ excretion into the cell wall and thus inhibited cell expansion and growth. ABA is known to be a general growth inhibitor (Milborrow, 1974), but also it is known, to inhibit chlorophyll accumulation in illuminated dark-grown tissue (Beevers et al., 1970). This inhibitory effect could be compounded by the accumulation of higher levels of ABA under water stress. K application to wheat in this study had very inconsistent effects on chlorophyll accumulation and growth. It is unlikely therefore, that the effect of this compound is explainable mainly in terms of a counter effect of, for example, an accumulation of ABA.

From the results reported here a number of conclusions can be drawn regarding the progress of greening in wheat leaf tissue:-

1. There was a marked effect of the age of wheat seedlings during greening and development on the accumulation of cellular components. This effect was most pronounced on the chlorophyll accumulation, where the capability of the accumulation was reduced with ageing. This effect was seen both in the age sequence along the leaf and in differently-aged seedlings.

2. Water stressing the leaf tissue had some effect on the pattern of greening in that chlorophyll accumulation was normally reduced, but this effect was most evident with intact seedlings.

3. The levels of chloroplast RNA fractions were high even in the dark-grown tissue as was the total NA content. Exposure to light led to an increase in the chloroplast RNA components.

4. Exposure to the higher water stress condition led to a loss of RNA components in the intact seedlings both in the chloroplast (consistent with inhibitory effects on chlorophyll accumulation) and also in the cytoplasm (consistant with inhibitory effects on growth, and potentially on greening).

5. Since the effects of water stress were most marked with the intact seedlings, it was apparent that the involvement of roots was important in the response of the leaves. This implies that there was some signalling between the roots and leaves, possibly through a plant growth regulator response, as reported by Mansfield et al. (1978); Davies et al. (1987) and Milligan and Dale (1988). However, even though cytokinins are shown to be involved in water stress, K did not counter the effects of PEG on wheat.

It is recognised that the experimental procedures for growth employed here were an exaggeration in terms of time span of growth in the dark. They seemed, however, to illustrate more clearly the changes which would occur under normal growth and greening patterns. The overall conclusions, therefore, are that growth and development of functional photosynthesis capacity of leaf tissue following growth in the dark is influenced by the age of the tissue and other environmental factors; in this study water stress. Any alteration of the capacity to green and the tissue to

develop the photosynthesis activity would have pronounced effects on the productivity of a plant under field conditions where seedling establishment is of great importance.

As noted in the General Materials and Methods statistical analysis of the results of the component extractions were not carried out. The results, therefore give an indication of trends in changes in the various components.

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Addendum

Generic and specific names of plants cited in thesis by common name

Alfalfa	Medicago sativa
Barley	Hordeum vulgare
Bean	Vicia faba
Bermuda grass	Cynodon dactylon
Birch	Betula pubescens
Blue grama	Bouteloua gracilis
Cabbage	Brassica oleracea
Carrot	Daucus carota
Creosote bush	Larrea tridentata
Crown gall bacterium	Agrobacterium tumefaciens
Cucumber	Cucumis sativus
Daffodil	Narcissus pseudo-narcissus
Maize/Corn	Zea mays
Oil seed rape	Brassica napus
Pea	Pisum sativum
Pumpkin	Cucurbita pepo
Radish	Raphanus sativus
Rice	Oryza sativa
Ryegrass	Lolium perenne
Scots pine	Pinus sylvestris
Soybean	Glycine max
Spinach	Beta vulgaris

Squash	Cucumis napus
Sugar beet	Beta vulgaris
Sugar cane	Saccharum officinarum
Sunflower	Helianthus annuus
Tobacco	Nicotiana tobaccum
Tomato	Lycopersicon esculentum
Western wheat grass	Agropyron smithii
Wheat	Triticum aestivum
Yeast	Saccharomyces cerevisiae