CHANGES IN β INHIBITOR AND CYTOKININ LEVELS IN RESPONSE TO LONG WILTING PERIODS IN GRAIN SORGHUM AT DIFFERENT GROWTH STAGES.

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CHAPTER ONE. LITERATURE REVIEW

1.1. INTRODUCTION

In the living plant, water occurs in many states; water of hydration and inbibition in colloidal phases such as cell walls, osmotic water in vacuoles and in phloem and hydrostatic water in the xylem. Water is involved in all physiological processes of the plant. Plant water status is a highly dynamic parameter and is influenced strongly by soil and atmospheric conditions. It is regulated to different degrees in different situations and with various species by physiological processes.

This review will be confined mainly to effects of water stress on:-

a) Several major physiological processes affecting growth and development.

b) Two main hormones (abscisic acid and cytokinins) and their importance in plant responses.

c) Grain yield in certain cereals.

1.2. Development of internal water deficits

Water deficit (stress) is defined as the state a plant enters when the water potential passes the zero mark and becomes negative. Scholander et.al.(1965) stated that the water potential in the xylem of many plants is negative most of the times. The actual water potential values depend on the two main factors: a) the level of the soil water potential and b) the diurnal lag of absorption over transpiration. Each of these factors, in turn, is influenced by other factors both environmental and physiological. Soil water potential is reduced progressively during a period of drought and there is a concomitant drop in level of plant water potential i.e. plant water potential cannot be higher than soil water potential. Hence there is a base level of plant water potential and internal water deficit which is limited by the level of soil water potential. Superimposed on this base level is the additional internal water deficit associated with the daily rhythm of transpiration and absorption (Slatyer 1967).

1.2.1. Parameters indicating plant water status

The parameters commonly used to indicate the degree of water status in plants are as follows:-

a) Water potential (Ψ) is now accepted and used as a basic measurement. It is expressed as energy per unit volume and with the

ABSTRACT

Plants of Sorghum bicolor L. Moench R.S. 610, a hybrid grain variety, were subjected to three water stress cycles during the following growth stages :-

(i) Late vegetative stage (ii) Boot to bloom stage

(iii) Dough stage.

Water status of the plants was measured by pressure bomb and Weatherly and Barr s' method. Both β inhibitor and cytokinin activity in leaves and roots were determined by two bioassays a) wheat coleoptile and b) radish cotyledon respectively. The bioassays results were expressed as (±) abscisic acid and kinetin equivalents respectively.

The results indicated that changes in levels and activity of ABA and cytokinin occurred in response to water stress. ABA levels in leaves increased rapidly up to 13 fold from the control to the maximum stress during the first and second stress cycles, while ABA levels of leaf samples from the third stress cycle only increased to 6 fold at the maximum stress period. In the roots , ABA levels did not increase markedly as the leaf samples in all the three stress cycles.

Cytokinin contents in both leaves and roots changed qualitatively and quantitatively in response to severe stress. There was a general decline in cytokinin activity as the magnitude of stress increased. There was a shift of cytokinin activity in peak 2 to peak 1 as the stress periods prolonged.

Grain yield, in terms of grain weight and grain number, was measured for the three stress cycles. The results showed that there were two 'critical' stages when sorghum plants were susceptible to stress that is a) late vegetative to early boot stage and b) during inflorescence development and flowering period.

The involvement of abscisic acid and cytokinins in the plant's adaptation to water stressed was discussed.

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chemical potential of pure water at atmospheric pressure and the same temperature as the reference point (Slatyer 1967). $\bar{\Psi}$ is the sum of the component potentials arising from the effect of solutes ($\bar{\Psi}_s$), of pressure ($\bar{\Psi}_p$) and matrix ($\bar{\Psi}_m$) such that:

$$\bar{\Psi} = \bar{\Psi}_p + \bar{\Psi}_s + \bar{\Psi}_m$$

b) Relative water content (RWC) is the water content relative to the water content of the same tissue at full turgor (after floating on water to 'constant' weight.

c) Tissue water content on a percentage of fresh weight.

Both RWC and tissue water content suffer a major shortcoming that is, both parameters are relatively insensitive indicators of mild stress (Barrs 1968)

Mild stress is considered to entail a lowering of plant ($\bar{\Psi}$) by several bar below corresponding values in well watered plants under mild evaporative demand. Moderate stress refers to a lowering of ($\bar{\Psi}$) by more than a few bar but less than -12 or -15 bar and stress would be severe if ($\bar{\Psi}$) is lowered more than -15 bar.

1.2.2. Problems in water stress studies

Slatyer (1972) reckoned that a clear and quantitative understanding of water stress effects does not exist, offering several reasons for this. Firstly, water stress affects almost all biophysical and biochemical processes, so the integrated effects on growth and development are extremely complicated. Secondly, plant water status is a highly dynamic parameter, strongly influenced by soil and atmospheric conditions. Finally, plant water status constitutes a difficult parameter to control experimentally.

1.3. WATER DEFICITS AND PHYSIOLOGICAL PROCESSES

The effect of water stress on such key processes as nutrient uptake, carbohydrate and protein metabolism, and translocation of ions and metabolites is closely linked with the effects on plant development. In turn, the effects are affected by other processes. For example, root development affects the size and character of the absorbing system for mineral nutrients; shoot development has a direct feedback on the rate of carbohydrate and protein metabolism.

Water stress affects the growth and development of plants in various ways, directly and indirectly.

1.3.1 Water deficits and plant development

The growth and development of a plant depends on continuing cell division ; the progressive initiation; differentiation and expansion of the cells until the characteristic form of the plant is realised (Slatyer 1969).

(A) Cell division This process shows a marked sensitivity to water stress, if stress is severe and protracted. For example, cell division may continue during stress at a much reduced rate until severe conditions occur (Kirkham et al. 1972). Cell division is related to cell size and cell number. Gardner & Nieman (1964) found that DNA increments per cotyledon of radish were reduced by more than half in the presence of -1 to -2 bar mannitol solutions during twenty-eight hours; little further reduction occurred with a lowering to -16 bar.

It is uncertain if the stress effect on cell division is direct; the effect can bepossibly via suppressed cell expansion. There is a possibility also of changes in growth regulators taking place during prolonged stress and thus affecting cell division.

(B) Cell enlargement This is the other essential component of growth and is affected at very slight stress levels (Hsiao et. al. 1970) and is usually the first observable symptom. In some species, cell enlargement is so sensitive to deficits that stem elongation and leaf growth can be inhibited by small diurnal deficits that occur even with well watered plants on days of high radiation incidence (Boyer 1968). Boyer (1970) demonstrated that when leaf Ψ dropped from -2 to -4 bar, leaf growth was inhibited by at least 75% in corn, soybean and sunflower in controlled growth environment. Photosynthesis per unit leaf area was only inhibited by 10% in corn and unaffected in the other two species. Boyer (1968) suggested that little growth occurs during the day when

leaf $\bar{\Psi}$ is rarely above -4 bar even in well watered plants; growth occurs primarily at night and this appears to depend on the length of time the leaf remains at high $\bar{\Psi}_w$. Boyer's result differs from Watt's recent finding (1974) which shows that leaf $\bar{\Psi}$ above -8 or -9 bar had little apparent effect on extension rate of Zea mays during vegetative stage in the field conditions. Watt concluded that the main reason for the apparent lack of sensitivity is due to $\bar{\Psi}$ gradients within the leaves.

A progressive decline in rates of cell enlargement is expected as water deficits develop, with enlargement ceasing when turgor pressure levels are still at several bar. Cell enlargement requires turgor to extend the cell wall and also requires a gradient in $\bar{\Psi}$ to bring water into the enlarging cell. This enlargement needs turgor to be above a minimum before irreversible enlargement occurs (Green et al. 1971). For rapidly growing vegetative plants, one of the first effects of drought is a reduced rate of growth by enlargement. The effect of reduction in cell enlargement decreases the total leaf area for photosynthesis. In general, cell enlargement appears to be more sensitive than cell division (Meyer & Boyer 1972) although Kirkham et. al. (1972) found an early effect of osmotic solutions on cell division.

The effect of water stress on the mature tissue or that approaching maturity, resembles that of hastened development, resulting progressively in protein hydrolysis and breakdown of normal cell functions. This hastens senescence which is irreversible, for plant tissues are unlikely to recover from a period of stress.

(C) Root development The overall growth of the root system or its increase in weight is determined by the availability of assimilate from the shoot in the vegetative stage with growth regulators being of lesser importance (Street 1969, Hatrick & Bowling 1973). The sources of this assimilate are the lower leaves on the stem (Rawson & Hofstra 1969), and of that assimilate translocated to the roots, possibly less than one third is used for growth; the balance is respired (Hatrick & Bowling 1973) or exuded into the soil (Barber & Gunn 1974).

Root growth is relatively insensitive to decreasing levels of soil water potential in the range down to -4 to -7 bar but below this point there is a marked reduction in most species of plants including cereals, cotton (Taylor & Ratliff 1969) and corn (Gingrich & Russell 1956). Lawlor (1973) found that growth of wheat roots stopped at -10 bar. The effects of water stress on root development include a reduction in rates of meristematic activity and root elongation and suberization so affecting the water and nutrient uptake properties of the root system.

1.3.2. Water deficits, photosynthesis (P/S) and carbohydrate metabolism

The photosynthetic capability of plants is determined primarily by the total leaf area and the activity of each unit of leaf. Water stress can have direct and indirect effects on (P/S) and on a number of intermediate components and processes.

In general, net (P/S) is reduced progressively by water stress, and negative values may develop when stress is severe. This response is mediated partly by way of impeded CO_2 supply following stomatal closure and partly by a direct effect of dehydration on the (P/S) system. Boyer & Mcpherson (1975) showed that at (LWP) of -18 to -20 bar, the (P/S) rate of maize was 15% of the controls. Boyer (1970) showed that in corn stomata appeared to shut partially whenever leaf Ψ decreased below -3.5 bar. For soybean desiccation had no effect on stomata until leaf decreased to -11 bar.

Non-cyclic photophosphorylation, cyclic photophosphorylation and electron transport through photosystems1 and 2 are reduced at leaf Ψ below -10 bar (Keck & Boyer 1974). In addition to the effects of low leaf Ψ on the photochemical portion of (P/S), there are also changes in some of the enzymes of the 'dark' reactions. Ribulose 1,5-diphosphate carboxylase activity is reduced when assays are performed on extracts from desiccated leaves (Huffaker et. al. 1970; Johnson et. al. 1974). However, none of these studies demonstrated an effect large enough to account completely for the inhibition of P/S in intact leaf (Jones 1973 ; Johnson et. al. 1974). Phosphoenolpyruvate carboxylase and ribulose-5-phosphate kinase also showed little change to be completely limiting (Huffaker et. al. 1970; Shearman et. al. 1972).

Thus, there is a general inhibition of a number of processes of the light reactions of (P/S). Boyer (1976) seems to think that the rates of (P/S) are limited either by stomatal effect or carboxylation activities or perhaps a combination of effects according to the conditions and the species involved.

The availability of suitable sinks for assimilates also influences (P/S) such that (P/S) is greatly reduced when assimilate utilization is impeded. During stress, assimilates probably accumulate at sites of (P/S), since leaf enlargement is restricted sooner and to a greater degree. Wardlaw (1967, 1971) examined this in wheat and concluded that although leaf (P/S) was not affected until growth rate had been reduced, there was no evidence of sink size directly affecting (P/S) rate. He postulated that the lack of suitable sinks could retard (P/S) under appropriate conditions.

(B) Water deficits and respiration This effect is difficult to determine; partly it is a problem of distinguishing between dark respiration and photorespiration and hence in measuring photorespiration. Part of the difficulty is due also to stomatal closure and the possible differences in short term and long term effects. In general, there was a progressive decrease in dark respiration of corn, soybean, and sunflower shoots as water stress increased (Boyer 1970).

The effect on photorespiration is difficult to evaluate because (P/S) is proceeding at the same time. A complication here is the marked dependence of respiration on temperature, since stomatal closure generally induces a rise in leaf temperature.

Another common effect of water deficits on carbohydrate metabolism, is an increase in sucrose levels and a decrease in starch levels. These are associated with reduced polysaccharide levels resulting from such factors as decreased (P/S) and increased hydrolysis as well as reduced synthesis. Hiller & Greenway (1968) concluded that reduced starch formation was an indirect result of increased sucrose synthesis, rather than a direct effect of stress on starch synthesis.

1.3.3. Water deficits, protein synthesis and nitrogen metabolism

In general, there is a close dependence of the growth rate of developing tissues and organs on protein synthesis and especially between protein synthesis and RNA and RNA and DNA levels. Nucleic acids are of major importance in cellular development and any effects of water stress on these compounds would be expected to elicit marked changes in the growth response of plants.

(A) Protein synthesis Shah & Loomis (1965) found that both soluble and total protein contents of sugar beet leaves declined progressively in a matter of days when water was withheld. This is attributed to either a retardation in synthesis or to an acceleration of degradation. Nir et.al.(1970) observed inhibition of amino acid incorporation in root apices which had been air dried at a controlled humidity, but only if water loss was more than 30% of the original fresh weight.

Dhindsa & Cleland (1975) demonstrated that water stress causes a differential inhibition of the synthesis of Avena coleoptile proteins, with the synthesis of some proteins being affected to a greater extent than the synthesis of others.

Polysome formation is slowed and breakdown occurs during water stress. This could be due either to a direct effect on the polysome themselves

or on the supply of m-RNA which is needed for polysome formation. Hsiao (1970) showed that water stress of etiolated maize seedlings caused a shift from polymeric to monomeric form of the ribosomes in rapidly growing meristematic tissues (a loss of 10% of fresh weight in 15 minutes). On rewatering, the ribosomes reverted to the polymeric form. Hsiao suggested that stress effects on protein synthesis are mainly at the translational level because of the rapidity of the response to stress and quick reversibility by re-watering.

Shah & Loomis (1965) found that DNA content per cell of sugar beet leaves was reduced by severe and protracted wilting, while RNA levels tended to decline at low stress levels. They concluded that RNA synthesis was impaired as well as some degradation of RNA. Gates & Bonner (1959) attributed the decline in RNA levels to enhanced degradation of RNA. The different responses observed by various workers are probably related in some degree to the developmental stage of the tissues and the methods of determination employed.

Itai and co-workers (1967, 1968) have postulated a key role for cytokinins in regulating protein synthesis during water and salinity stress. This view is based on the reduction in cytokinins in leaves by stress (see page 30) and on the ability of applied kinetin or benzyladenine to alleviate a part of stress-effected reduction in amino acid incorporation or in leaf protein content.

In rapidly growing tissue, protein synthesis appears to be readily and reversibly reduced by very mild water stress. The dynamic responses to stress and stress release may be controlled at the translation level. Hsiao (1973) reckoned that the basis for the response is still obscure.

(B) Proline and other amino acids Total free amino acids in leaves often are increased if severe water stress lasts several days Barnett & Naylor (1966). Amides frequently increase (Barnett & Naylor 1966) but proline has the most marked rise (Palfi 1968; Routley 1966; Singh et. al. 1973a). In sorghum (Sorghum bicolor L.) leaves of water stressed field grown plants accumulated proline to a level several times greater than in non-stressed plants (Waldre et. al. 1974). Upon leaf rehydration after stress, proline content decreased rapidly to pre-stressed levels. Proline is due to protein hydrolysis (Palfi et. al. 1966) and may be oxidized as a source of energy especially when the carbohydrate content is low (Oaks et. al. 1970).

Singh et al. (1973b) indicated that barley varieties which accumulated more proline tend to survive extreme water stress more readily and grew more rapidly following relief from the stress. They suggested

the possible roles are (1) to neutralize toxic free ammonia produced in water-stressed leaves and (2) to serve as a substrate for respiration and energy source for the recovery of the plant following stress.

1.3.4. Water stress and enzyme levels and activity

Todd (1972) listed some twenty-five enzymes affected by water deficits. The effects of moderate to severe stress on enzyme levels are as follows:

a). enzymes involved in hydrolysis or degradation usually remain at the same level or increase ; they do not decrease until fairly severe desiccation has taken place.

b). severe stress generally causes an overall decrease in enzyme level.

c). levels of some enzymes involved in synthesis are decreased and levels of others increase.

Mattas and Pauli (1965) observed nitrate reductase decreased in activity early in the drying cycle when little change in leaf relative water content occurred and at the same time nitrate accumulated in the plants.
The level of phenylalanine ammonia-lyase was found also to decrease with mild to moderate water stress and to recover readily with rewatering (Bardzik et.al.1971). Hsiao (1973) suggests that because nitrate reductase and phenylalanine ammonia-lyase both have short half lives and respond rapidly to water stress, and hence suppressed protein synthesis could account for their decreased activity.

α-amylase and ribonuclease in leaves are observed to increase with a moderate to severe water stress. The functional significance of these increases remains obscure (Hsiao 1973).

Some enzymes that are involved in the photosynthetic pathway are not easily reduced in activity by mild stress (Hsiao 1973).

1.4. Abscisic Acid And Cytokinins In Plants

Introduction

Plant growth hormones play a central role in the internal control mechanisms of plant growth, interacting with key metabolic processes such as nucleic acid and protein synthesis. They may either enhance or diminish growth rate, depending upon their nature and concentration. The auxins, the cytokinins, and the gibberellins are generally regarded as growth stimulators, whereas both ethylene and abscisic acid often inhibit growth. These growth regulators are concerned in the overall growth rate and in the correlation of growth activities by acting as chemical signals. They move from one cell, tissue, or organ, to another and thereby provide a means of communication between different parts of the plant.

This review will cover abscisic acid (ABA) and cytokinins, and examples of their interaction in growth processes.

1.4.1. Abscisic acid and its phenomenon in plants

Abscisic acid is the trivial name for 3 methyl-5(1'-hydro, 4'2'6'6' trimethyl 2'-cyclohexen 1'-yl)- cis, trans-2,4- pentadienoic acid. The natural abscisic acid is referred to as (+)ABA and the synthetic as (±) ABA. Generally, ABA in literature refers to the natural (+)ABA. Figures 1 and 2 are cited geometric isomers of ABA.

Bennet-Clark & Kefford (1953) first detected the inhibitor β- complex which is an unpurified fraction of one dimensional chromatograms and is inhibitory in some bioassays. Milborrow (1967) stated that ABA is the most active component of the inhibitor β complex. There are indications that quantitative differences in inhibitor β contents reflect the quantitative variations of ABA (Wright & Hiron 1970). However, in potato tubers, the inhibitor β consists of several compounds including an active phenolic substance (Holst 1971). With pea root extracts, the inhibitor β consists of at least three compounds including trans-cinnamic acid and ABA (Tietz 1971). Most results suggest that ABA is the principal and dominant growth inhibitor of the complex.

ABA was first isolated from young cotton fruit by Ohkuma et al.(1963). The structure was confirmed by Cornforth et. al.(1965) by synthesis. In most plants, the naturally occurring isomer of abscisic acid is cis-ABA. Trans-ABA is much lower in content and less active biologically than ABA and its presence might due to light induced conversion from cis-ABA (Dorffling 1971).

Another naturally occurring substance related to ABA is abscisyl-glucopyranoside (Figure 3). This was first isolated from immature fruits of Lupinus luteus (Koshimizu et.al 1968) and seems to be widely distributed in plants. Milborrow (1970) showed that synthetic ABA applied to tomato shoots was metabolized to this compound. Moreover, the excessively high ABA content in wilting plant tissue was accompanied by an increased content of the glucoside in spinach (Spinacia oleracia L.) shown by Zeevaart (1971). This glucoside has the function of a 'bound' reserve form of ABA and has inhibitory activity when hydrolysed to 'free' ABA (Osborne et al. 1972).

(A) Occurrence and metabolism of abscisic acid

ABA is widely distributed in higher plants, and has been detected in at least forty species. Relatively high amounts have been found in fruits, resting seeds, buds, tubers and in senescent and wilting leaves. The hormone level varies in relation to growth and developmental processes. Davis et.al.(1968) showed ABA changes during cotton fruit development; for example a rise in ABA was correlated with young cotton fruit abscission and with the senescence and dehiscence of the mature fruit.

Abscisic acid has been reported to occur in extracts of roots of Lens culinaris (Fries et al. 1971) and Zea mays (Kundu & Audus 1974a, 1974b ; Wilkins & Wain 1974). Other related inhibitory substances have also been found in root tips, including xanthoxin (Kundu & Audus 1974a). According to these authors, ABA is associated primarily with the root cap and xanthoxin is associated possibly with the 'root meristem'. ABA was extracted from seedling roots of Vicia faba by El-Antably & Larsen (1974) and demonstrated by gas-liquid, thin-layer chromatography of purified samples.

The presence of ABA in xylem exudate was reported for Salix viminalis by Lenton et.al.(1968) and for Helianthus annuus by Hoad (1975).

(i) Biosynthesis of ABA

ABA is synthesized in several different parts of plants. Leaves, fruit and seed tissues (cotyledons, endosperm, embryos) have been shown to incorporate labelled mevalonate into ABA (Milborrow et.al. 1973). Two schools of thought of biosynthesis of ABA are, briefly, (1) that ABA is derived from a carotenoid, in particular violaxanthin, by photolytic cleavage or oxidative processes; and (2) that ABA is synthesized by a direct route from mevalonic acid.

Xanthoxin (Figure 4) had been detected as a photooxidation product

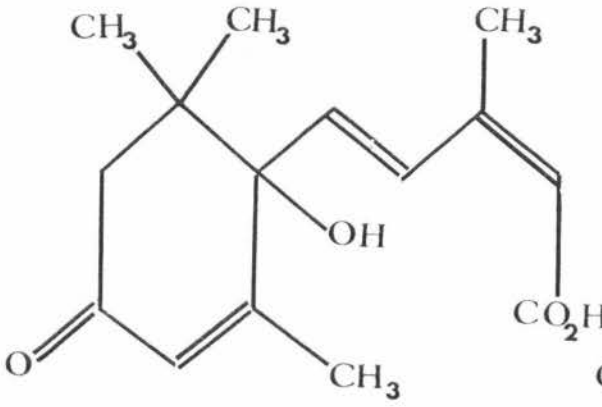

Figure 1. (+)-Abscisic acid (cis-isomer)

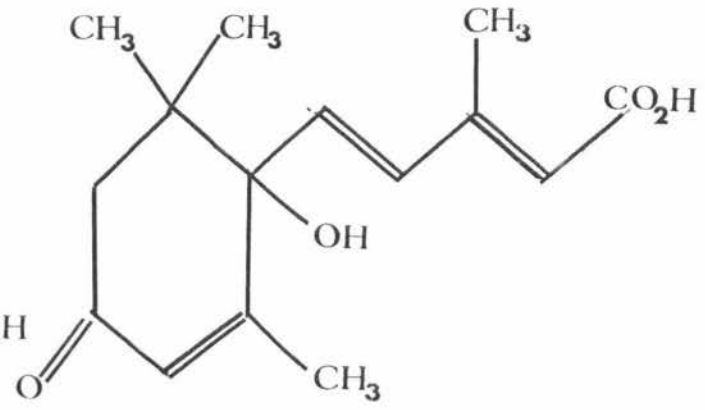

Figure 2. 2-trans-abscisic acid (t-ABA)

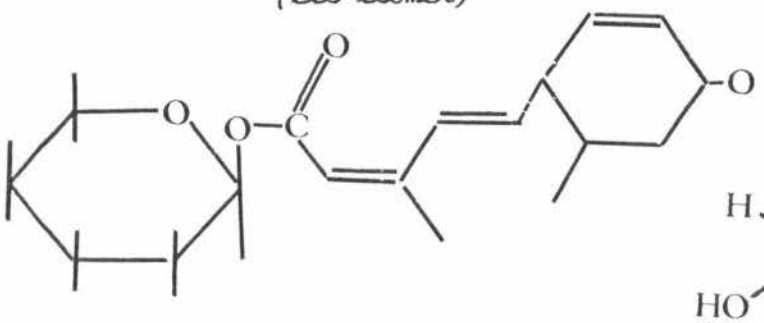

Figure 3. +(abscisyl-B-D-glucopyranoside)

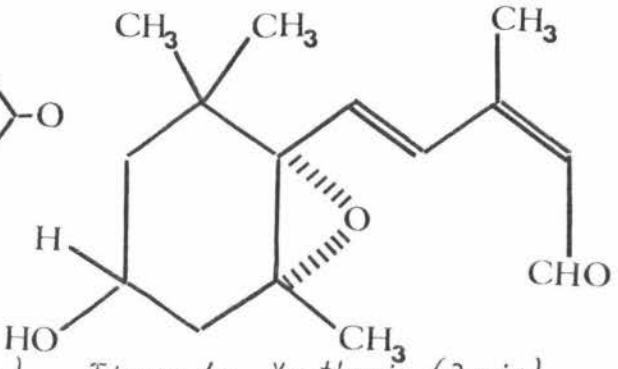

Figure 4a. Xanthoxin (2-cis)

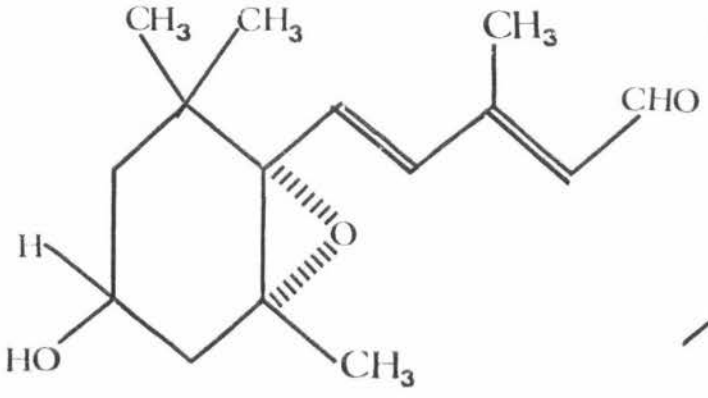

Figure 4b xanthoxin (2-trans)

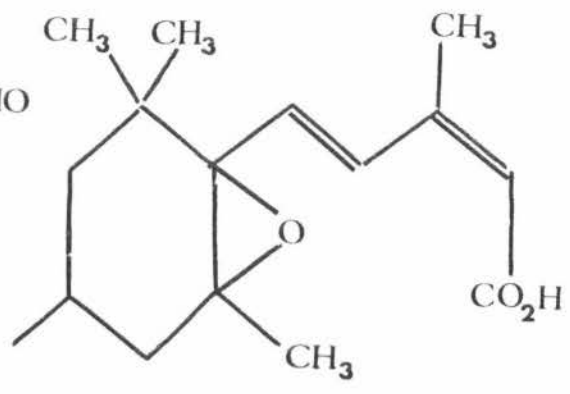

Figure 5 1'2'-epoxy-B-ionylideneacetic acid

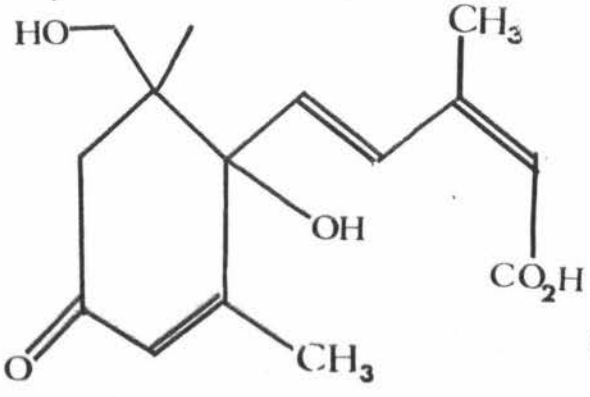

Figure 6 metabolite C

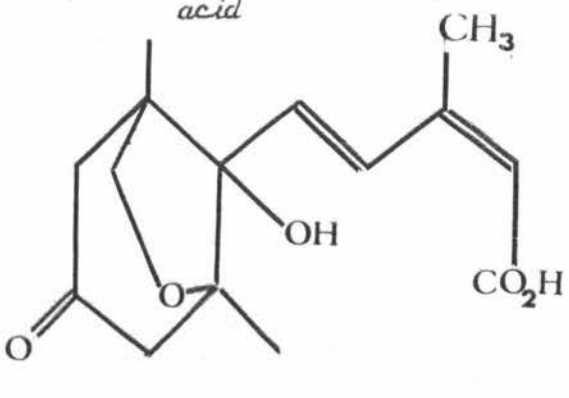

Figure 7 Phaseic acid

of xanthophylls, especially from violaxanthin (Taylor & Burden 1970). It has been found in several plants (Firn et.al.1971) and can be converted chemically into t-ABA (Burden & Taylor 1970). The yield of xanthoxin ; both cis and trans-xanthoxin from violaxanthin is low and high light intensities are required for photolysis in vitro (Milborrow 1974). Recently, Firn & Friend (1972) have reported that in soybean (Glycine soja) lipoxygenase is capable of cleaving violaxanthin oxidatively to form similar products , in similar yield, to that formed during photolysis. The possibility that xanthoxin is produced by the action of lipoxygenase removes the requirement of light for its production. Milborrow (1974) reckoned that xanthoxin in the leaf is an adventitious product formed by light and is unlikely to be a precursor of the cis-isomer of abscisic acid.

Milborrow & Noodle (1970) investigating the second possible route of biosynthesis and found that H^3- mevalonic acid was converted to ABA via an epoxide (Figure 5) which may be a direct precursor of ABA. Wilting tissue which has an excessively high amount of ABA, converted nine times more mevalonic acid than non-wilting tissue.
The main pathway of ABA biosynthesis seems to be a direct one, and the degradation of certain xanthophylls to ABA might be of less importance.

Rogers et.al.(1966) have demonstrated that there are two pools of terpenoid biosynthesis, chloroplastic and extra-chloroplastic from fruits. ABA has recently been characterised from chloroplasts of Pisum species by GC- MS (Railton et.al.1974) and is ,in all probability biosynthesised in chloroplasts of avocado (Persea gratissimia) Milborrow 1974.

(ii) Degradation of ABA

Milborrow (1970) reported that (2-^{14}C) labelled abscisic acid was converted to three products: 'Metabolites A, B and C'. Metabolites A and B were identified as methyl abscisate and abscisyl-β-D-glucopyranoside respectively. This glucose ester (Figure 3) had been characterised by Koshimizu et.al.(1968) as a major metabolite and appears to be a rapid storage product for extra ABA. This compound has been identified in citrus by Goldschmidt (1973).

Metabolite C (Figure 6) was isolated in crystalline form and had the same physical properties as phaseic acid (Figure 7). This metabolite could undergo an internal nucleophilic attack of a hydroxyl group on a double bond, to give phaseic acid. Phaseic acid had been isolated from bean seeds (Phaseolus vulgaris) by Macmillan et al (1968), and found also in grape vine (Vitis vinifera L.) by Lovey & Kriedemann (1973).

(iii) Transport of ABA Like the rates of biosynthesis and degradation, the patterns of hormone transport are of great importance in determining the hormonal environment, and hence growth rate of individual cells distant from the source of the hormone.

Using the donor-receiver system with petiole and stem segments of Coleus and cotton, ABA was shown to be transported very quickly within the parenchymatous tissue with a velocity of 24 to 36 mm per hour in Coleus and 20 to 30 mm per hour in cotton petioles (Dorffling and Bottger 1968 , Ingersoll and Smith 1971). Transport in young Coleus petioles was mainly basipetal, whereas in older tissues of Coleus nearly no polarity was observed. In cotton petioles, however, no polarity was found. Milborrow (1968) noted also a basipetal polarity of 3:1 in bean sections.

Ingersoll and Smith (1971) reported that ABA transport is reduced by DNP (2,4-dinitrophenol), low oxygen tension and low temperature (2 C). The transport of ABA through explants is considered to be a metabolically controlled cell to cell tranfer rather than phloem transport because the sections are too short for sieve tubes to function. The rates of ABA transport are considerably slower than those calculated for intact phloem (100 to 1500 mm per hour)

(B) Plant responses to abscisic acid

Broadly, there are two major groupings: responses of inhibition and promotion. Much of this section will emphasize the role of the plant hormone as correlation factors in plant responses.

(i) Senescence Decreases in the levels of chlorophyll, protein and RNA are the prominent symptoms of leaf senescence. Back & Richmond (1971) ABA applied to leaves promotes senescence which may support the proposal that ABA functions as a senescent factor in leaves and also favours the increased synthesis of degradative enzymes. For example, ABA treatments have increased the activity of chromatin-associated ribonuclease in excised barley leaves (Srivastava 1968) and in bean endocarp (de Leo & Sacher 1971). ABA also accelerates the synthesis of cellulase even beyond the rate inducible by ethylene (Lewis & Varner 1970).

Leaf senescence is a complex and tightly regulated process and it is intimately coupled with the development of the plant as a whole. This process also involves other hormones like cytokinins and auxins. Cytokinin-like substances produced by the root delay leaf senescence on intact plants (Sitton et al. 1967; Wareing & Seth 1967). The hormonal regulation in senescence is possibly mediated through a control

of DNA dependent RNA synthesis.

(ii) Abscission ABA stimulates abscission in excised leaf explants of citrus (Altman et al. 1971), bean, cotton (Cracker et al. 1969), and Coleus (Bewley et al. 1972). Diffusates from senescent Coleus petioles accelerate abscission, whereas diffusates from young petioles delay abscission. Cracker et al. (1969) found that ABA treatments increased ethylene production and cellulase activity in cotton and bean explants. Ethylene has been known to be a very potent abscission accelerator. During abscission, there is also a rapid increase in pectinase (Morre 1968) and peroxidase, dehydrogenases and phosphatases (Sutcliffe et al. 1969). Such changes can be presumed to be promoted by ABA as a consequence of its promotion of abscission.

(iii) Growth inhibition The inhibition of the growth of whole plants, excised organs or seeds is the most easily measured response to ABA. ABA inhibits growth of all parts of plants and counteracts the stimulatory effects of the natural growth promoting compounds when applied with them. Hsiao (1973) and Dorffling (1972) noted ABA retards internode elongation. Pilet (1972) determined that the inhibitory action of ABA on cell elongation appeared to be due to an antagonistic effect on auxin.

This growth inhibition is remarkable in three ways:

a) It is extremely potent, being of the same order as the other natural regulators (0.05 to 0.5 ug/ml gives a 50% response in most tests (Aspinall et al. 1967)
b) It can be reversed by removal of the source or by leaching of the tissues.
c) ABA counteracts the toxicity of supra-optimal concentrations of the growth promoting substances (Milborrow 1966).

It is difficult to demonstrate whether endogenous ABA inhibits normal growth mainly because ABA can be biosynthesized in several parts of the plant and there is no known inhibitor of ABA biosynthesis yet.

Inhibitory substances including ABA are present in correlatively inhibited lateral buds (Dorffling 1964). Inhibitor β and synthetic ABA can inhibit out-growths when applied to the lateral buds (Blumenthal et al. 1965). Apart from direct growth inhibition, ABA could act as a 'negative effect' on nutrient accumulation, and hence result in apical dominance. For example, in sunflower seedlings, decapitated below the primary leaves and treated with low amounts of ABA (0.2 ug/ml per plant) a decrease of potassium and phosphorus levels was observed in the epicotyl.

(iv) Dormancy The induction of bud dormancy is one of the well known effects of ABA and was observed first by Eagles & Wareing (1964). Application of ABA also prolongs dormancy. The hormonal regulation of bud dormancy, at least of terminal buds, may operate as follows. Accumulation of ABA at the growing apex of the plant leads to inhibition of internode growth and the formation of resting buds. The release from bud dormancy is caused by a decrease of the level of ABA-like substances and increase of the level of gibberellin and auxin-like substances. Low temperatures and photoperiod may be factors which influence the release from dormancy (Tinklin & Schwabe 1970).

(+) ABA is a potent inhibitor of seed germination, and is regarded as the major growth inhibitor in dormant seeds of many species (Aspinall et.al. 1967 ; Khan 1969 ; Sondheimer et.al. 1966). ABA has the role of maintaining the state of seed dormancy. Khan et.al. (1971) have proposed a working hypothesis of seed dormancy and germination according to which gibberellin is the primary stimulus for germination whereas the roles of endogenous cytokinins and inhibitors (ABA) are 'permissive' and 'preventive'. The special role of cytokinin is to remove the block to germination caused by the inhibitor and allows the gibberellins to complete their stimulative action.

Mechanisms by which ABA accumulates in buds and seeds, could be by synthesis in these organs or translocation to the respective organs from other parts of the plant. Wareing et.al. (1964) shown that leaves of sycamore produce more ABA-like inhibitors under short conditions than long days. These inhibitors are probably transported in the phloem sap to the growing apex, where they accumulate. However, Lenton et.al. (1971) have pointed out '' the conclusion that shortdays lead to a rise in ABA levels must remain open to question until such a rise has been demonstrated by direct and specific measurements on the ABA content of extracts ''.

(v) Abscisic acid and water relations

The level of ABA in leaves can be raised by a variety of conditions, wilting (Wright 1972a , 1972b), waterlogging of the root system (Wright 1972a), low relative humidity (Mizrahi et.al. 1971), osmotic stress (Mizrahi et.al. 1970), lack of mineral nutrients (Mizrahi & Richmond 1972), cold stress (Milborrow & Robinson 1973) and infection with the wilt-inducing bacterium Pseudomonas solanacearum (Steadman & Sequeira 1970). A common feature of these factors is that they affect the water balance of plants.

Wright & Hiron (1972b, 1973) found that when cut shoots were wilted, there was an increase in β inhibitor complex. They identified the major inhibitor as (+) ABA and defined the conditions under which the increase occurred. A water loss of about 10% of the total fresh weight causes an approximately 40 fold increase in the ABA content. A key feature is the rapidity of the increase in ABA. The authors calculated the ABA content of turgid dwarf bean leaves as initially 6 ug/kg rising to 9 ug/kg within 7 minutes of the warm air treatment; 33 ug/kg by 25 minutes and 68 ug/kg within 45 minutes. Wright & Hiron (1973) observed that both the level of conjugate and of free ABA increased considerably. A similar dramatic rise in ABA content on wilting has been found in Brussel sprouts (Wright & Hiron 1970), peas (Dorffling et.al. 1974, Ivy 1974); sugar cane (Most 1971); wheat (Milborrow et.al. 1970); tomato (Rammussen 1976), maize (Beardsell & Cohen 1974), sorghum (Beardsell & Cohen 1975, Ogunkanmi et.al. 1974) and tobacco (Boussiba & Richmond 1976).

The rapid increase in ABA contents in wilted leaves remains fairly constant until the loss of turgor is regained. Milborrow (1974) reckons that there are probably three switch mechanisms operating:-

a) rapid synthesis triggered by wilting,
b) a stop message when sufficient ABA has been formed and
c) either the beginning of degradation of ABA or the cessation of rapid synthesis.

Milborrow & Robinson (1973) suggested that the excess ABA from the 2(^{14}C) diols-ABA operated a negative feedback loop and stopped its own biosynthesis. Evidence tends to suggest that the rapid increase in ABA is not due to release from a 'bound' form but to synthesis from mevalonic acid (Milborrow & Noddle 1970).

Zabadal (1974) working on two species of Ambrosia, observed that there is a threshold water potential that stimulates ABA synthesis. The ABA increase at the threshold water potential is so abrupt that a reduction in leaf water potential of only one atmosphere may cause a significant rise in ABA from its baseline level. Beardsell & Cohen (1975) confirmed that there is a threshold value of (LWP) below which ABA levels increased abruptly in maize. Cummins (1973) and Milborrow (1974) provided some evidence that ABA occurs in discrete compartments in the cell. One postulate is that when (LWP) reaches the threshold value, a re-distribution of ABA occurs. The reduction in ABA at sites of accumulation might in turn act as the trigger for enhanced ABA synthesis.

Loveys & Kriedemann (1974) demonstrated that moisture stress in grape vines (Vitis vinifera L.) contributing towards an increase in stomatal resistance (R_s) was correlated with increase levels of endogenous ABA and phaseic acid in mature foliage. Phaseic acid has been identified as a by-product from degradation of ABA (Gaskin et.al. 1973).

(vi) Abscisin acid and stomata The closure of stomata by ABA is an important role first observed by Mittelheuser & Van Steveninck (1969). Horton (1971) ; Jones and Mansfield (1970) have also showed that exogenously applied ABA has a dramatic effect on stomatal aperture. Application of (±)-ABA at 1 ug/ml concentration caused stomatal closure in leaves of wheat and barley and also reduced transpiration by one half. Talha & Larsen (1975) studied the ABA effect on transpiration of Zea mays. A linear relationship occurred between the transpiration rate of both detached and intact leaves and the concentrations of applied ABA. The magnitude and persistence of the treatment effect depended on the concentration of ABA and the species of plant used. Jones & Mansfield (1970) reported that the decrease of stomatal aperture induced by 10^{-4} M ABA in intact leaves of Xanthium strumarium persisted for up to nine days after application. In contrast to Jones and Mansfield s' result of slow stomatal recovery, Kriedemann et.al.(1972) have observed quite rapid recovery from ABA treatment. This may have been due to the fact that they applied approximately ten times more hormone than would have been required to close the stomata; the excess may then have acted as a 'reservoir'. Fischer et.al.(1970) noted that bean leaves show stomatal recovery in 1 to 2 days following water stress.

The effect of ABA on the transpiration rate may be discussed in relation to the following possibilities:

a) A direct action of ABA on the stomatal apparatus (guard cells)
b) Biochemical changes induced by ABA.
   i) ABA has been shown to interact with other hormones in several hormonally regulated plant responses including the antagonistic effect of ABA and cytokinin on stomatal opening (Imber & Tal 1970; Mizrahi et.al.1970).
   ii) Leshem(1971)found that ABA significantly inhibited the production of RNA by activation of the enzyme RNase.

Changes in endogenous levels of ABA-like substances can constitute a mechanism for regulating gas exchange. These may occupy a key position in the regulation of photosynthetic performance. Prolonged closure of stomata inhibits photosynthesis and possibly reduce the yield of crop.

Kriedemann et. al.(1972) suggested that a doubling of the endogenous level of ABA is sufficient to initiate stomatal closure in well watered bean plants. Cummins et.al. (1971) pointed out the rapidity and ready reversibility of the ABA action on stomata and suggested that is is a good modulator of stomatal behaviour. However, Hsiao (1973) questioned whether the ABA accumulation from stress is fast enough for it to be the modulator of stomatal responses. Stomata possibly close even faster than the increase in ABA.

Mansfield & Jones (1971) showed that ABA treatment results in starch accumulation in the chloroplasts and a fall in the osmotic potential of guard cells from 14.1 to 9.8 bar. There was also a flux of K^+ out of the guard cells. It was also shown that ABA prevented the accumulation of potassium in guard cells, and increased the starch content of their chloroplasts. The authors suggested that ABA could act on the K^+ flux and causing stomatal movement; perhaps via an osmotic adjustment involving starch hydrolysis.

Raschke (1975) observed that the simultaneous requirement of CO_2 and ABA for the modulation of stomatal aperture in Xanthium strumarium. It appears that ABA reversibly blocks the active secretion of H^+ from guard cells. In the presence of CO_2, this would lead to a rapid acidification of the cytoplasm and to stomatal closure. In many species, the intracellularly evolved CO_2 may suffice to elicit acidification. However, in species with strong H^+ pumps, malate formation would lead to acidification only in the presence of ABA. This possibly explains the sensitization of stomata to CO_2 by ABA. Note that CO_2 is needed as a substrate for the acidification of the cytoplasm as well as the production of osmotica. The mechanism whereby ABA is able to regulate stomatal aperture remains unclear. A direct effect in terms of enhanced acidification by inhibiting expulsion of H^+ from guard cells is an attractive hypothesis but is still hypothesis but is still regarded as speculative.

(C) Mode of action of ABA

As with some other plant hormones, ABA has probably several primary sites of action:-

i) Hormonal effect on the enzyme synthesizing apparatus.

This includes DNA itself or any of the factors participating in transcription or translation. ABA causes specific inhibition of RNA synthesis. Addicott (1970) showed that the action on nucleic acid metabolism is largely that of inhibiting or modifying the synthesis of one or more major fractions of RNA. It may inhibit activity of the enzyme RNA polymerase in transcription process. The transcription block thus formed prevents synthesis of DNA-primed RNA. Bex (1972) detected a marked decrease in the specific activity of RNA polymerase when maize coleoptiles were incubated with ABA. However, Leshem (1971) suggested that the overall decrease in RNA levels is due to ABA activation of the enzyme RNase and not necessarily by inhibition of RNA polymerase. The mode of RNase activation by ABA is difficult to verify (Leshem & Schwartz 1972).

Inhibition of DNA synthesis (replication) by ABA has been observed also by van Overbeek et.al. (1968) and Walton et. al.(1970). However Haber et.al. (1969) showed that ABA inhibits lettuce (Lactuca sativa) seed germination in circumstances which do not involve DNA synthesis. DNA synthesis might not be a primary effect: often this inhibition does not closely correlate with ABA's physiological effects such as increased ABA levels in response to wilting. Further, there is evidence of a dual effect of ABA in some physiological processes such as senescence and abscission, simultaneously inhibiting some aspects of nucleic acid metabolism and promoting others. The effects of ABA on DNA and RNA have to be interpreted with care (Addicott et. al. 1969; Dorffling 1972).

There is often a marked lag in time between ABA treatment and observed changes in nucleic acids suggesting that the effects may be indirect and secondary.

ii) Abscisic acid and allostery

Another possible mode of action may be via the regulation of the enzyme activity by allosteric promotion and inhibition (Saunders & Poulson 1968) without directly affecting transcription and translation. This would mean that ABA acts on a 'preformed system' leading to observable responses without a lag period. ABA may serve as a negative allosteric effector on biologically

active proteins which may include enzymes participating in GA biosynthesis , and on DNA polymerase. van Overbeek et.al. 1967 have proposed an overall scheme for ABA action as a ' negative' allosteric effector (Figure 8)

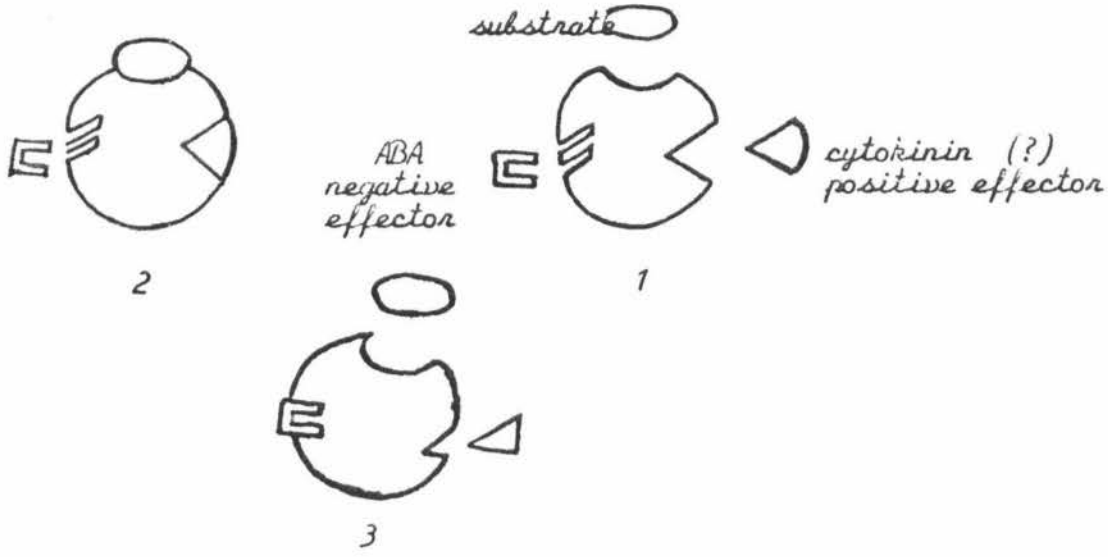

Fig. 8 The allosteric effect of ABA (After van Overbeek et.al)

The 'positive' effector or activator in their model may be more specific i.e. cytokinin. It is postulated that ABA attachment to the protein is by means of its polar groups which link to specific 'allosteric sites' situated on the protein (stage 3). The attachment of ABA causes a steric change of the 'substrate site', preventing the entry of the substrate and thus inhibiting biological activity. If the activator e.g. the promoting hormone, is attached beforehand (stage 2); it lends stability to the protein and prevents deformation of the 'substrate site' and thus the attachment of the ABA. Biological activity can then proceed normally. This model outlines how in vivo, growth may be the function of an equilibrium attained between promoting and inhibiting factors.

Another important primary action of ABA seems to be an effect on membrane properties. ABA acts on specific ion uptake mechanisms such as potassium, phosphorus and chloride (Reed & Bonner 1974 ;Cram & Pitman 1972 ; Dorffling et al. 1972). The uptake of potassium , for example is reduced by ABA within 30 minutes in coleoptile sections, and the inhibition reaches a maximum of 75% within 2 hour. There is no simple relation between the inhibition of growth and the inhibition of ion uptake. An effect of ABA on the permeability of plant cells and roots as regards water is a controversial matter (Cram & Pitman 1972; Glinka 1973).

There are therefore, two kinds of responses to ABA, a direct ,rapid response possibly operating on membranes or some other structure, and a slower effect involving the synthesis of new enzyme protein.

1.4.2. CYTOKININS AND PHENOMENA IN PLANTS.

Cytokinins are compounds which promote cell division in cultured plant cells (Skoog et al. 1965). They are growth substances which are usually derivatives of the nucleic purine base, adenine. The common synthetic cytokinins include kinetin (6-furfuryladenine) and BAP (6-benzyl aminopurine). Endogenous cytokinins are zeatin (6-(4-hydroxy-3-methylbut-trans-2-enyl) aminopurine and IPA (N^6-iso-pentenyladenine). Zeatin occurs naturally not only as the free base but also as its nucleoside and nucleotide (Miller 1965; Letham 1966a, 1966b). Both IPA and zeatin constitute by far the most active cytokinins yet discovered. These or closely related compounds have been detected in a diverse range of plants (Kende 1971).

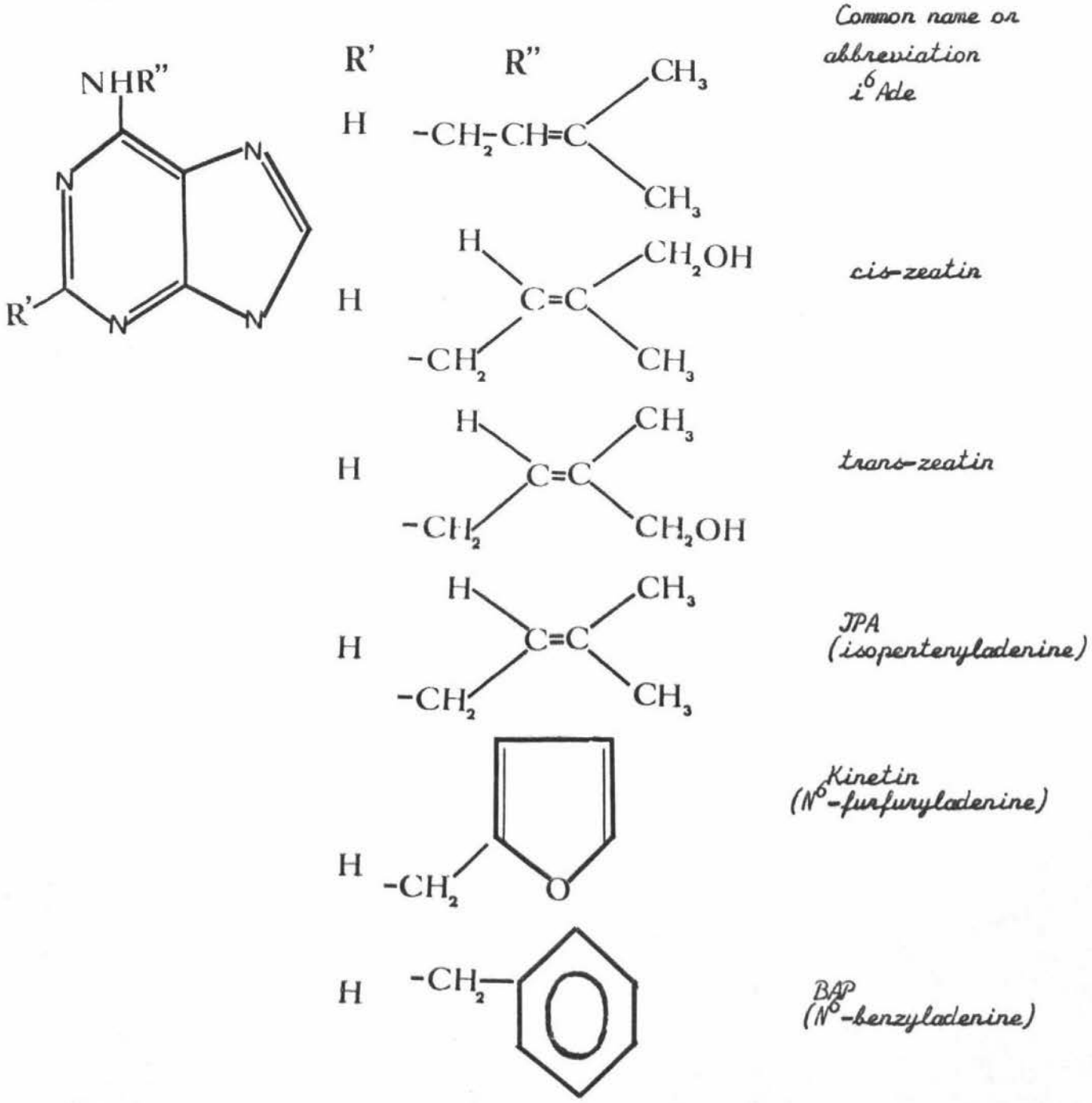

Figure 9 Structures of common cytokinins.

A) Occurrence of cytokinins

Kende (1964, 1965) showed that xylem sap of sunflower contained cytokinins and substances with cytokinin activity have now been detected in xylem sap in many species. Cytokinins in xylem seem to resemble the common cytokinins detected in other plant parts for example leaves. Recently, trans-zeatin riboside was identified positively in sycamore sap by combined gas liquid chromatography- mass spectrometry (Horgan et al. 1973). They suggest that zeatin riboside, the free base and the nucleotide are present in xylem sap. Skene (1972) identified zeatin and zeatin riboside in xylem sap of the grape vine (Vitis vinifera).

Most evidence suggests that cytokinins are of root origin. Substances with cytokinin activity have been detected in extracts of roots of sunflower (Weiss & Vaadia 1965); grape vines (Skene 1970); peas (Short & Torrey 1972a); rice (Yoshida & Oritani 1971) and Solanum andigena (Woolley & Wareing 1972). Radin & Loomis (1971) found increasing amounts of three cytokinin fractions in developing roots of radishes. Two of these were chromatographically similar to zeatin ribotide and to zeatin or its riboside and the third fraction was not identified.

Evidence strongly favours the meristematic regions of the roots as sites of synthesis. Weiss & Vaadia (1965) found that in sunflower seedlings, free cytokinins were confined to the youngest portions of the root tip. Short & Torrey (1972a) examined cytokinins in both the free form and as constituents of transfer RNA in serial segments of young seedling roots of pea. The greatest amount of cytokinin existing in a free form and present in t-RNA was distributed in the highly meristematic 0 to 1 zone of the mm root tip. Smaller amounts were found in those segments 1 to 5 mm behind the root tip. Zeatin and its derivatives and an unidentified cytokinin are the active free cytokinins. Short & Torrey suggested that the 'quiescent zone' and the surrounding meristematic tissues as centres of cytokinin production in the root tip of pea seedlings. Recently, Feldman (1975) working on intact terminal millimeter root tips of Zea mays shows that at least four cytokinin fractions are present, that is nucleotide, zeatin and zeatin riboside and unidentified cytokinin.

(i) Cytokinins in leaves and other plant parts

Cytokinins in leaf tissue are present in diverse forms. Hewett & Wareing (1973b, 1973c) detected at least seven cytokinins in mature leaves of Populus X robusta. The three major cytokinin fractions were zeatin and zeatin riboside and a cytokinin glucoside. These findings

agreed with Engelbrecht (1971) who suggested that zeatin and its riboside are the main cytokinins present in rooted leaf cuttings of Phaseolus vulgaris L. The physiological significance of the diverse forms of cytokinins in leaf tissue remains unclear.

High levels of cytokinins are also present in other meristems, such as cambial tissue, young fruits and seeds (Zwar et.al. 1963 ;Letham 1966 ;Letham & Williams, 1969). Indirect evidence suggests that the seed does produce a significant proportion of its own cytokinin (Blumenfeld & Gazit 1971) and it is quite clear that a range of cultured tissues are capable of synthesizing cytokinins (Miura & Miller 1969; Short & Torrey 1972b).

(ii) Metabolism of cytokinins. The rate of metabolism is of great importance in determining the hormonal environment where growth may be regulated. So far, little is known really about the internal and external factors which determine these processes. The metabolism of cytokinins differs markedly from plant to plant species and also from plant organ to plant organ. However, some common features are apparent.

When plant tissues are supplied with cytokinin, the bulk is degraded by a process which cleaves the side chain. The purine ring is converted subsequently to several metabolites. A smaller part of the applied cytokinin remains intact and exists as the free base or as ribonucleoside or ribonucleotide (Tzou et.al. 1973) or as glucosides (Parker & Letham 1973 , 1974; Fox et.al. 1973). A 7-glucose of zeatin ('raphanatin') has been detected in derooted radish seedlings which had been supplied with ^{3}H-zeatin (Parker & Letham 1973). It is present only in the cotyledons, not in the hypocotyl, petiole and xylem sap of this plant. It occurs also in small amounts in the roots of Zea mays (Parker and Letham 1974). In general, the cytokinin glucosides seem to be very stable in the plant and raphanatin is biologically active. The physiological significance of naturally occurring cytokinin-glucose complexes in root and other tissues is yet to be elucidated. It seems likely that they are storage compounds for excess cytokinins.

It is possible that plant tissues make use of t-RNA or other metabolic pathways as a source of cytokinins, depending on situations. The breakdown of the t-RNA can be a potential source of release of certain fractions of cytokinins. Chen & Hall (1969) indicated that cytokinins in t-RNA are synthesized by the attachment of the isopentenyl group to preformed t-RNA. It is also presumed that mevalonate is the precursor of the isoprenoid side chain of free cytokinins. Short & Torrey (1972a)

showed that there was approximately 27 times more free cytokinin than the amount detected in t-RNA in root apices of pea. The turnover of cytokinins from t-RNA is less rapid. These two aspects tend to indicate that cytokinins in root tips are produced by biosynthesis seperate from the catabolism of t-RNA.

(iii) Translocation of cytokinins Cytokinins synthesized in the root tips are translocated to the above ground parts where they regulate the protein metabolism of leaves and other aspects of shoot development. Levels of cytokinin activity are generally quite high in the xylem of woody species of plant, suggesting that substantial quantities of cytokinins pass in the transpiration stream. However, little is known about the actual amounts moving in the transpiration stream. Hewett & Wareing (1973a) suggest that the riboside is the usual form of cytokinins transported in the xylem of woody species.

Developing organs seem to compete for available root cytokinins: for example, limited supplies of root cytokinins move preferentially to the apex. Pilet (1968) showed that the removal of the apical bud inhibits the acropetal transport of (^{14}C) BAP to stems of Lens culinaris. Application of labelled kinetin to the roots of intact plants results also in activity accumulating in the apical bud itself. Morris & Winfield (1972) suggest that hormone-directed transport of cytokinins is involved in the regulation of lateral bud growth contending some component of cytokinin movement in the shoot is under directional control.

Parker & Letham (1973) reckoned that zeatin ribotide accumulation in hypocotyls of rootless radish seedlings resulted from lateral movement from the transpiration stream rather than from basipetal transport from the upper parts of the seedlings. On the other hand, experiments with ^{14}C BAP indicate that BAP applied to the base of rootless cuttings of Solanum andigena accumulates rapidly in the leaf and later is redistributed to other parts of the cutting (Woolley & Wareing 1972b). They are of the opinion that auxin influences the distribution of cytokinins from roots. Auxin applied to the cut upper surface of Solanum andigena cuttings prevents the outgrowth of lateral buds and inhibits the accumulation in the lateral buds of label from basally administered ^{14}C-BAP. In the absence of auxin, labelled cytokinin accumulates in the lateral buds prior to their growth as leafy shoots.

Skene (1975) points out that not enough is known whether cytokinins from roots move directly to the apex and other centres of high auxin concentration, or whether they are re-exported from the leaves.

(B) Biological activity

Cytokinins are known to influence a wide range of physiological and biochemical processes. As well as being involved in all phases of plant development; from seed germination to plant aging, senescence and cell division and organ formation. These processes are not dependent on one single hormone but rather a concerted, correlated regulatory action of several growth hormones.

(i) Mitosis and cell division The property most characteristically associated with cytokinins is their stimulation of cell division in plant tissue cultures. The presence of cytokinins in meristematic tissues such as root tips, cambium, and fruitlets has been taken as indication that cytokinins are involved in regulating cell division.

Guttman (1956) presented data showing that kinetin acted sometime during the interphase of mitosis to trigger the subsequent prophase in root cells of onion (Allium cepa). Torrey (1961) with intact roots of the garden pea (Pisum sativum cv. Alaska) obtained a somewhat similar results. Here, cytokinins brought into division mature cells in the root cortex which normally may undergo chromosome doubling but rare mitosis or cell division.

Cytokinins are widely referred to as regulators of cell division and distribution of activity often follows the intensity of cell division in the tissues. Letham (1963) reported a correlation between cytokinin content and the rate of cell division in apple and plum fruitlets. There is a definite correlation between the rate of tissue growth and level of measurable cytokinin in xylem sap of grape vine (Skene 1972).

(ii) Cell enlargement Cell enlargement in discs of etiolated leaves was increased markedly in the presence of kinetin and certain analogues active in cell division (Miller 1956; Scott & Liverman 1956). Arora et. al. (1959) reported that cortical cells of tobacco roots enlarge up to four times their normal size in the presence of kinetin. Glasziou (1957) demonstrated that under certain low kinetin concentrations, an increase in cell elongation of tobacco pith occurred. Kinetin treatment of sunflower hypocotyls caused an increase in fresh and dry weight more than double that of the controls although no elongation occurred.

(iii) Morphogenesis Cytokinins are certainly involved in the formation of organs which takes place under appropriate conditions in a variety to tissue cultures. Cytokinins regulate the development of organs from callus. The auxin/ kinetin ratio of the culture medium was the

critical factor in determining the type of organ: that is bud and root (Skoog & Miller 1957). In this case cytokinins have the ability to modify the effects of other hormones for example auxin. Jordan & Skoog (1971) in their study with coleoptile tips of Avena sativa L. showed that i^6-Ade and BAP stimulated synthesis of auxin. Cytokinins do not act alone in plant regeneration processes. They interplay with other growth regulators particularly auxins delicately and quantitatively.

Cytokinins have been implicated also in a large variety of systems which involve differentiation in one way or another; in the differentiation of tracheids through activation of lignin biosynthesis (Bergmann 1964) ;in the induction of parthenocarpy in certain fruits (Weaver et al. 1965) and in the maturing of proplastids into plastids (Stetler & Laetsch 1965). There have been no unifying concepts offered to explain adequately a host of morphogenetic responses to cytokinin treatment.

There is too, a correlation between rate of tissue growth and the level of measurable cytokinin activity. In developing cotton fruit, and avocado seeds, the level of cytokinin activity is much higher during the early stages of development, and as the tissue matures, the cytokinin level also falls (Gazit et al. 1970; Sandstedt 1971).

(iv) Dormancy Dormancy is shown by a wide range of plant organs, of very different morphology for example buds and seeds. The available experimental evidence for dormancy can be grouped into three categories. Firstly, there are observations which indicate that in particular cases, a transmissible 'hormonal' stimulus must be involved in the imposition or removal of dormancy. Secondly, it is possible to discern a parallel variation in the endogenous level of a particular hormone and the state of dormancy of a plant organ. Finally, exogenous hormonal treatments can impose or break dormancy.

Cytokinins, notably kinetin and benzyladenine, are effective in overcoming dormancy of tree buds, tubers and other resting organs (Vegis 1964; Wareing & Saunders 1971; Weaver 1963). Bud dormancy may be controlled by a balance between endogenous inhibitors such as ABA and the growth promoting hormones especially especially gibberellins and cytokinins for the cytokinin-inhibitor antagonism can be an essential ingredient of hormonal regulation (Khan 1971).

Cytokinins can promote seed germination for Miller (1956) showed that cytokinins can substitute for the red light requirement in breaking seed dormancy. Germination of seed or growth of an organ is often a result of cumulative actions of several hormones, each having a designated role and not always a result of an increase or a decrease in the

level of promoters and inhibitors (Khan 1971). The mechanism of cytokinin-inhibitor interaction in seeds and buds has been studied at the level of nucleic acids and enzymes. The following possibilities can occur:

a) ABA and benzyladenine showed opposite effects in ^{32}P incorporation into all nucleic acid fractions (van Overbeek et. al. 1967).
b) The nucleotide composition of labelled RNA was altered by ABA and this effect was reversed by kinetin (Khan et. al. 1970).
c) ABA and cytokinins interact at the level of translation and enzyme synthesis. For example, α-amylase synthesis in intact cereal grains which is mediated by gibberellins from the embryo is inhibited by ABA and reversed by cytokinins (Khan & Downing 1968).
d) ABA can affect a decrease in the degradation of kinetin to adenine, (Back et. al. 1972) supporting the suggestion of Mullins and Osborne (1970) that ABA has a 'cytokinin sparing' effect. It has been suggested that this could stimulate a plant process.

(v) Senescence Cytokinins from the roots appear to be responsible for maintenance of balanced protein metabolism in leaves. Richmond & Lang (1957) showed that the protein level in kinetin-treated, detached leaves declined more slowly than in untreated controls, and a similar effect of kinetin on RNA levels was observed by Osborne (1962). Osborne found that incorporation of a labeled amino acid into protein, and of $^{32}PO_4^{3-}$ into RNA, was enhanced by treating detached leaves with kinetin. Similar results have been obtained by other researchers using different plants (Gunning & Barkley 1963; Kuraishi 1968; Tavares & Kende 1970). Tavares & Kende concluded that cytokinins retard senescence of corn leaves primarily through inhibiting proteolysis and possibly RNA breakdown.

The activity of proteases (Beevers 1968; Atkin & Srivastava 1969) and of RNases (Sodek & Wright 1969) is lower in cytokinin-treated leaves than in the corresponding controls that have not been treated with the hormone. In some plants cytokinins depress the activity of protease and RNase temporarily rather than inhibit an increase in the activity of these hydrolases.

Cytokinins retard the decrease in levels of DNA, RNA and protein and also stabilize or even promote RNA/DNA ratios, thus indicating that RNA metabolism may be directly involved. One hypothesis is that cytokinin acts by preventing the synthesis of specific m-RNA coding for

degradative enzymes. ABA is involved also in the regulation of senescence in leaves (see page 13).

Sitton, Itai & Kende (1967) found that the cytokinin content of xylem sap of sunflowers increased during the exponential growth phase, and decreased rapidly when growth ceased and flowering commenced. The decrease in the supply of cytokinins from roots to leaves is one of the factors leading to senescence. Growing fruits can take over as sites of cytokinin synthesis when the supply from the roots decline. Cytokinins may be diverted however to developing fruits at the expense of leaves (Wareing & Seth 1967).

The onset of senescence is seen to be a complex interaction between organs of plant, a part of this interaction being mediated by cytokinins of root origin and ABA.

(vi) Miscellaneous effects of cytokinins The list of cytokinin activities is now too large to include here but the following appear to have potential significance.

Cytokinins can prevent partially the toxic effects of certain phytopathogens. For example kinetin reduces the number and size of lesions caused by tomato spotted wilt virus (Selman 1964) and antagonizes the the toxic effect of Pseudomonas tabaci which causes 'wildfire' disease of tobacco (Lovrekovich & Farkas 1963).

Cytokinins are involved in the regulation of photosynthetic enzymes. They are required for the development of the following enzymes of the Calvin cycle: JuDP carboxylase, NDP^{+} dependent glyceraldehyde dehydrogenase, transketolase, and ribosephosphate isomerase (Feieraben & Pirson 1966). Application of kinetin to dark-grown seedlings enhanced the activity of ribulose 1,5diphosphate and NADP-dependent glyceraldehyde phosphate dehydrogenase up to the level found in illuminated seedlings. Removal of the roots as a cytokinin source reduced the activity of both enzymes, and this reduction could be overcome by kinetin treatment. Changes in cytokinin level appeared to affect preferentially, chloroplast enzymes.

Cytokinins seem to promote the synthesis of chlorophyll and development of the chloroplasts. Etiolated seedlings lose the ability to produce chlorophyll and soluble proteins with increasing age and kinetin retards this process (Stobart et. al. 1972).

Syano and Torrey (1976) showed the presence of high cytokinin activity in root nodules of pea (Pisum sativum L.) and the cytokinins found predominantly in pea root nodules infected by Rhizobium leguminosarum were zeatin and its riboside and ribotide. The presence of cytokinins in root nodules and their changing concentration during nodule develop-

ment support strongly the idea that cytokinins play an important role in nodule development, particularly in relation to the meristematic nature of the nodule that is its cell division activity and also to the distinctive polyploid state of the dividing nuclear population.

(C) Cytokinins and factors affecting production

Cytokinins synthesis by roots is influenced by factors of the environment that either directly or indirectly affect root physiology. These factors are discussed below:

i) pH; Nutrition Low pH of the root medium adversely affects the growth of many plants. Cytokinins could be detected in xylem sap of maize plants grown in medium of pH 7.0 but not pH 4.0. (Skene 1975). The nutritional status of the plant also affects cytokinin production by roots for example reduced quantities of cytokinins were found in xylem sap and root extracts of sunflowers (Wagner & Micheal 1969, 1971), and in root extracts of Solanum andigena (Woolley & Wareing 1972c) when grown in media of low nitrogen levels.

ii) Root temperature Skene & Kerridge (1967) found qualitative differences in the cytokinins of xylem sap from sultana vines (Vitis vinifera L.) grown at root temperatures of 30 C and 20 C, a cytokinin nucleotide being absent from the 30 C samples. They also showed an increased export of cytokinins from the roots of plants grown at the higher temperature. This is associated with a larger root system and a higher bleeding rate.

iii) Waterlogging This reduces the growth of the shoots and induces chlorosis of the lower leaves, symptoms which may be related to reduced quantities of gibberellin (Reid et.al.1969) and cytokinins (Burrows & Carr 1969) moving from root to shoot. Burrows & Carr (1969) showed that the decline in cytokinin content of sunflower sap was paralleled to a decline in metabolic activity of the root apices after three days of flooding.

iv) Photoperiod Van Staden & Wareing (1972) demonstrated that cytokinins in xylem sap of short day plant, Xanthium strumarium appear to predominate in the nucleotide form when grown under long day condition. However, the nucleotide fraction decreases after exposure to short days ; while the free base and riboside fraction remained virtually unchanged. It is not clear whether differences in the cytokinin content of mature leaves of Xanthium strumarium under long and short days are due to an effect of daylength on cytokinin synthesis within the leaf, or are a result of interconversions within the leaf of cytokinins imported from the roots.

v) Season Luckwill & Whyte (1968) reported that the cytokinin content of xylem sap from apple stems remained low during winter, increased in early spring, reached a peak level about the time of full bloom, and thereafter decreased. The authors suggested that the disappearance of cytokinin activity coincided with the cessation of extension shoot growth which may be associated with cytokinins in the sap. Increases in cytokinin activity at the time of budburst are also suggestive of a causal relationship between cytokinins and budburst. (Note changes in the balance of other growth regulators in the bud are implicated too.)

vi) Water stress Various stresses applied to the root system result in physiological changes reminiscent of the aging process for example the decline in levels of proteins and RNA, as well as affecting the quantities of cytokinins moving from root to shoot.

Itai & Vaadia (1965, 1969) suggested that stress reduces the levels of cytokinins in xylem sap in sunflowers and reduces the capacity of tobacco leaf discs to incorporate(^{14}C)L-leucine into protein (Ben-Zioni et.al. 1967). Itai & Vaadia (1971) demonstrated with tobacco that less than 30 minutes of wilting reduced substantially the cytokinin activity in root exudate. The decrease was reversible, and upon termination of the water stress, cytokinin activity increased again. The adverse effects are partially counteracted by cytokinins. Pretreatment of stressed tobacco leaf discs with kinetin partially restored incorporation of amino acids into protein. It is suggested that stress induced a decline in leaf protein synthesis resulting from a deficiency of cytokinins in the leaves. Although in both root and shoot stress, cytokinins levels are similarly reduced, the manner in which the reduction occurs may be different. It is possible that under shoot stress inactivation of cytokinins present may be more common than under root stress.

Reduced cytokinin transport from stressed roots can result in disturbed leaf performance and especially affects stomatal response. Vaadia & Itai (1965) showed that cytokinins promote stomatal opening and thus enhancing transpiration rates, an effect which seems to be of a general nature. Cytokinins in association with other hormones such as ABA may modify plant response to stressed conditions by altering stomatal opening and permeability of plant tissues to water (Livne & Vaadia 1972 ;Mizrahi & Richmond 1972). ABA is a major factor facilitating the adaptive response of plants to root stresses that impede water balance, whereas the ratio ABA/cytokinins is implicated in directing the extent of the response.

(D) The mode of cytokinin action

The occurrence of cytokinins in certain t-RNA's from a wide variety of organisms has led to much speculation that these plant hormones may exert their physiological effects through regulation of protein synthesis, by modifying the synthesis and function of specific t-RNA s (Anderson & Cherry 1968). Hall and co-workers (1967) have isolated both 2-IPA and ribosyl-zeatin from plant t-RNA hydrolysates ; while wheat germ t-RNA contains at least four cytokinins identified as ribosyl-zeatin, 2-iPA and their 2-methylthio-derivatives (Hecht et. al. 1969).

IPA, the natural cytokinin, is present in t-RNA for the amino acid serine, isoleucine and tyrosine and within the adaptor molecule located adjacent to the anti-codon. One theory (Leshem 1973) is that cytokinin action is associated with codon-anticodon recognition on the mRNA. The alternative approach is presented by Kende & Tavares (1968) who stated that cytokinin action is not dependent upon its presence in t-RNA. They proposed that IPA is not formed from cytokinin but that initially adenine exists as an integral part of the t-RNA molecule and to this an isopentenyl side chain is attached. The breakdown of t-RNA results in nucleotide release which migrates to other cellular sites where they exert their function as growth regulators.

For the present, it is difficult to know the actual mode and sites of cytokinin action. Like other plant hormones, they can certainly influence the rate of nucleic acid metabolism and protein synthesis, and these effects often parallel the effects of the same substances on growth rate. Higher plants also exhibit a very great flexibility in their growth response to a range of environmental influences. Hence, they must possess a very complex internal system of controls which are amenable to environmental variations.

1.5. Effect of water stress on grain yield in cereals.

In simplest terms, yield is related to the production of total dry matter. The situation is more complex when the yield is considered as only a part of total plant material that is, as grain or storage tissue. Then, the yield will depend more on the developmental stages at which stress is applied and on the sensitivity to stress at those different stages.

Three key stages in the grain formation and crop yield are:

1. Floral initiation and inflorescence development stage where the potential grain number is determined.
2. Anthesis and fertilization stage, when the degree to which this potential realized is fixed.
3. Grain filling stage, when grain weight increases progressively.

There are in addition, considerable differences in morphogenesis and reproductive development between various cereal species.

1.5.1 Inflorescence development Slight water stress can reduce the rate of appearance of floral primordia. Husain & Aspinall (1970) showed that number of primordia in barley is more sensitive to water stress than devlopment of existing primordia. This is probably typical for most cereals. They suggest that if the stress is mild and relatively brief, rate of primordial initiation, upon relief of stress, is more rapid than in the controls and the total number of spikelets formed may be unaffected. On the other hand, if the stress is severe, or protracted, total spikelet number may be reduced substantially. Nicholls & May (1963) suggested that the number of spikelets per inflorescence in barley is determined by the balance between the rate of primordial initiation relative to that of spikelet development. Since spikelet development is less affected by stress than primordial formation, it follows that prolonged stress at the stage of floral initiation could markedly reduce the potential number of grains per ear.

Whiteman & Wilson (1965) found that the development of the sorghum inflorescence could be suspended during stress, yet could be resumed on re-watering and result in a flowering head not significantly different from that of control plants. In general, stress applied prior to panicle initiation merely delays panicle initiation without greatly influencing yield per plant in sorghum.

Hultquist (1973) investigated the influence of water stress on yield components at panicle initiation, at floret differentiation, and at early

grain fill of two hybrid genotypes, C-42Y and RS 626. A severe reduction about 60%, in seed number per head resulted from stress imposed during panicle initiation. No primary panicle branch loss was noted when the stress came during early grain fill, but there was a 25% loss in seed number through floret abortion. Seed size compensation following the 60% seed number loss from the stress applied at panicle initiation was about 30%. There was no seed size compensation following the early seed fill stress. Hultquist (1973) suggested that differentiation of spikelet components is a critical period to water stress, and seed number can be reduced drastically at this stage. Photosynthesis may also be limiting during seed number differentiatiation and during subsequent development.

From the stage of spikelet initiation to fertilization of the ovules, a number of other processes, associated with the development of the inflorescence, are likely to be sensitive to water deficits and thus cause a reduction in the number of grains per ear, or even in the number of fertile ears. Water stress interferes specifically with the sexual development of the spikelets, such as meiosis of the gametes in barley (Aspinall et. al. 1964) and in wheat (Chinoy 1962). However, the availability of mineral nutrients and carbohydrates during the preflowering phase also appear to influence spikelet development, floret fertility and grain set.

In wheat, the potential for variable floret numbers provides an opportunity for compensatory effects if stress is removed. This may also apply to oats, but would not apply to crops such as rye, barley or maize in which floret number is fixed (Bonnett 1966). On the other hand, although compensation may not occur, relief of stress in many species during the stage of inflorescence development may permit final grain number to approach potential represented by the number of spikelets initiated.

1.5.2. Water stress and fertilization Stress at anthesis markedly reduces fertilization and grain set in most cereals. Corn is the most sensitive at this stage; with a 50% reduction in yield by brief periods of wilting (two or three days) Denmead and Shaw (1960). It is suggested that stress at this stage acts either by way of dehydration of pollen grains or by impairment of growth of pollen tube. Robins & Domingo (1953) were of the opinion that stress interferes with the germination of the pollen tube from the stigma to the ovules in corn.

Fisher (1972) states that grain yield in wheat is reduced most when the stress develops about ten days before ear emergence, because of pronounced effects on the grain number formed per spikelet.

1.5.3. Water stress and grain filling Ultimate grain yield depends on the rate of dry matter accumulation and the length of the grain filling period. Grain yield is a function of an efficiency component and a time component. The efficiency component is complex, being influenced by sink adequacy and a network of physiological processes (see page 3 to 8). Further, some factors influencing the efficiency component may also influence the time component.

Grain weight is influenced both by pre-flowering and post-flowering conditions. In almost all cases, the post-flowering stage is the more important. Yield development requires photosynthate accumulation in the grain. The two sources for these assimilates are (P/S) in the ear itself and translocation from other parts in the plant. Although photosynthate accumulated prior to anthesis contributes to grain filling, by far the most contribution comes from the ear, the leaves and stem (P/S)(Carr and Wardlaw 1965; Allison and Watson 1966). Asana (1966) showed in wheat, that nearly all the increase in dry weight after anthesis is associated with grain filling.

Prolonged stress through out grain filling, even at moderate levels, reduces grain weight. Fischer & Kohn(1966)have shown that wheat yields tend to be inversely correlated with the stress induced rate of senescence of photosynthetic tissue after flowering. There is more rapid senescence of older leaves which could lead to a flow of assimilates from them towards the ear (Allison & Watson 1966) as in maize.

Wardlaw (1967, 1969) has shown that there is little effect of water stress on translocation of assimilates in the conducting tissue itself. He has pointed out that translocation out of the leaves is slowed and prolonged by water stress. This phenomenon, combined with evidence that water stress hastens maturation, and with the direct effect on (P/S) in the ear and leaves, contributes to lower grain weight in stressed plants.

Slatyer (1972) stated that the relative importance of (P/S) in the ear, flag leaf and elsewhere in grain filling does not appear to be a major factor in interpreting yield decrements under water stress. There are important differences between species associated partly with crop morphology for example the role of ear (P/S) is greater in wheat than in corn (Allison & Watson 1966).