Studies On Certain Physiological & Biochemical Processes Dnring Seed Germination Usiug Cycloheximide

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By

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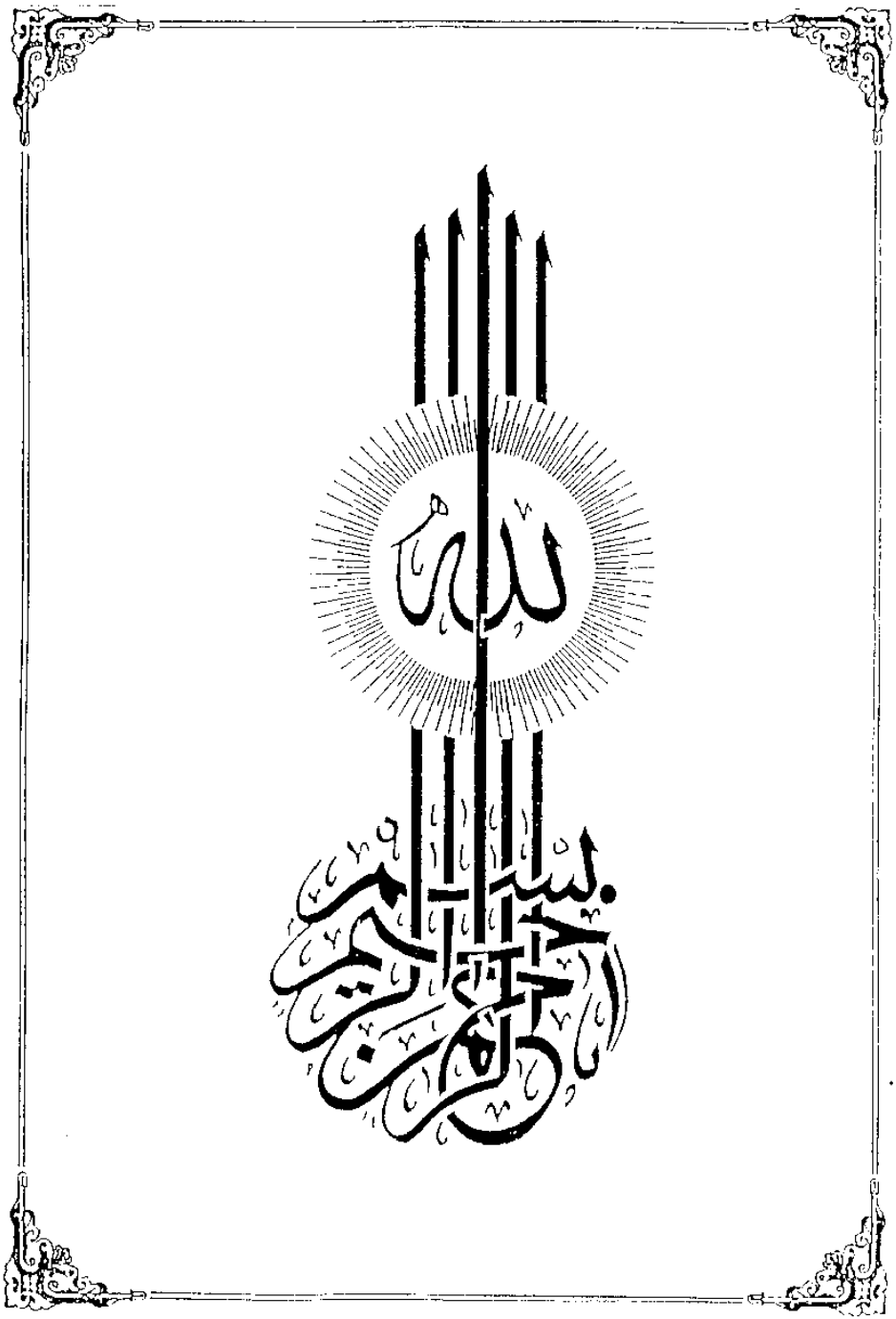
DEDICATED
TO MY SMALL FAMILY
AND BIG ONE



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This thesis has not been previously submitted for any degree at this or at any other university.

The references in the text will show specifically the extent to which I have availed myself of the work of other authors

Magda Mahmoud Ibrahim Abdo

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INTRODUCTION AND AIM OF WORK

INTRODUCTION

Germination is probably the most vulnerable stage in the life history of a plant when it is most subject to stress, predation and infection (Osborne et al., 1977). The biochemistry and physiology of seed germination is currently regaining great interest. Although studies in this connection are relatively vast and numerous, there are still key problems concerning those mechanisms which control some of the metabolic sequences involved. Interest has undoubtedly been more recently stimulated by the complementary binding that a ligand and receptor is the basic language of intercellular communication (Chadwick and Garrod, 1986). It is probably fair to say that this has hampered research on plant hormone receptors. Such unsolved problems need integrity of research work in biochemistry and molecular genetics (Libbenga et al., 1986). In particular, there is hope that more positive results can be gained from studies on seed germination to know more about the integration of metabolic activity and control mechanisms since germination is less complicated and more limited than growth and differentiation.

In this connection, Yamaki and Kobayashi (1970) suggested the existence of some kinds of sRNA capable of binding IAA. The genesis of this type of sRNA is apparently a biological phenomenon

because it is produced only when IAA-^{14}C is supplied to living tissues. The formation of the sRNA which binds IAA-^{14}C is observed within 30 minutes after the supply of IAA-^{14}C and the sRNA becomes saturated with IAA at about 2 hrs after incubation. The amount of this sRNA is directly correlated with, and may regulate the growth rate. Native auxin is isolated from specific parts of sRNA on BD-Benzoylated diethyl amino ethyl cellulose column where fed IAA-^{14}C is always located. Recently, the presence of soluble hormone-binding proteins and RNA species is often regarded as essential for a cell to respond to a hormonal signal especially when the response includes alterations in expression of the genome (Libbenga et al., 1986). The main question is which biochemical reaction or process is regulated by the receptor in the presence of physiological concentrations of the hormone. According to this criterion, Chadwick and Garrod (1986) stated that only two IAA-binding proteins can be classified as putative receptors, both modulating RNA-polymerase activity in the presence of IAA. The first was isolated from immature coconut endosperm nuclei and the second from cultured tobacco tissues (Libbenga et al., 1986) and both were regarded as a first step to prove an involvement of this protein in the auxin-mediated regulation of transcription. How does such binding phenomenon relate to the biological action of the ligand ? This of course is equally true of putative receptor systems associated with other plant growth regulators such as gibberellins (Stoddart, 1986),

cytokinins (Fox and Erion, 1975), and ethylene (Bengochea et al., 1980). When proteinaceous receptor sites are under investigation, it is imperative that they are distinguished from those of enzymes metabolizing the compound in question (Stoddart, 1986).

Growth regulating substances are known to induce alterations in RNA and protein metabolism in all kinds of responsive tissues (Carr, 1970; Cherry, 1977; Jacobsen, 1977; and Libbenga et al., 1986). This is not surprising since cell division, cell extension, and cell differentiation, which are major processes controlled by auxin and other growth substances are closely connected with RNA and protein metabolism. However, the problem is how directly phytohormones are involved in the control of these long-term processes ? , and at which level of the information stream: DNA $\longrightarrow$ RNA $\longrightarrow$ protein, they possibly act ? .

It is also generally known that protein synthesis by tissues is a pre-requisite for auxin action on cell responses for light (Carr et al., 1970; and Poulson & Beevers, 1970) and extension growth (Cleland et al., 1970; Masuda et al., 1970; and Reinhold & Ganot, 1970).

Thus, in the present study, cycloheximide as an inhibitor of protein synthesis has been used at properly inhibiting dose as

well as at relatively low stimulatory concentrations to affect the germination of wheat grains in an effort to know more about how the control of the accompanying processes is exerted and whether protein synthesis is the key step for hormonal activity and other metabolic contributions or not during seed germination. Throughout the present work there is less emphasis on specific processes and more concern about how processes interrelate in the intact tissue. In the following pages, the effects of cycloheximide on germination and main accompanying processes will be dealt:

Seed germination

Choudhuri et al. (1978) found that cycloheximide inhibited germination of *Ruellia* seeds which are positively photoplastic in nature. Dark inhibition of germination could be counteracted by GA_3 replacing light requirements. Amylase activity of seeds was promoted by treatments which accelerated germination and was inhibited by cycloheximide. Nucleic acids had insignificant role in this process. Grubisic et al. (1978) studied the effect of several antibiotics and base analogs on germination of light stimulated *Paulownia tomentosa* seeds. They showed that only cycloheximide in concentrations of 10 μg/ml or higher inhibited germination completely. Tlaskal (1979) utilized the inhibiting action of

cycloheximide (70 ppm) on cell mitosis in combination with 8-hydroxyquinone (250 ppm) as a pretreatment of the root meristems of *Zea mays*. The preparations contained nearly ten times more cells in prophase that were suitable for chromosome counting than those given a single pretreatment with 8-hydroxyquinone alone. This new pretreatment has been developed especially for chromosome counts and studies in tropical grasses with a large number of small chromosomes. Tsoneva and Chakalova (1980) found that the reduced germination percentage of rye seeds by cycloheximide (μg/ml) was accompanied by reduced plastid size particularly that of etioplasts but no obvious effect on plastid structure could be observed. Bose et al. (1982) showed that cycloheximide inhibited germination of maize seeds and protease activity in the endosperm. Senaratna and Mckersie (1983) showed that cycloheximide (20 μg/ml) inhibited the germination of soybean seeds on studying their sensitivity to dehydration changes.

Protein synthesis

The literature in this connection is relatively vast. Thus, Davies and Exworth (1973) studied the nature and the dose response paradox of the inhibition of protein synthesis brought about by cycloheximice. Burguillo and Nicolas (1977) utilizec cycloneximice as

a protein inhibitor to study the nature of an alternate cyanide-resistant respiratory pathway in germinating *Cicer arietinum* (chic pea) seeds. The results indicated that this patnway is dependent upon cytoplasmic protein synthesis. Chenieux et al. (1977), using cycloheximide, proved that protein synthesis is a requirement for the action of auxins, GA, cytokinins, and growth inhibitors. Jones (1977) got evidence that cycloheximide acts as a glutamine analog in plant tissue. This author indicated that ^{14}C-asparagine formation in growing maize root tissue is inhibited and ^{14}C-glutamine accumulation is stimulated as a result of cycloheximide application. He concluded that the alteration in amide metabolism following cycloheximide treatment is a direct result of the antibiotic acting as a glutamine analog. Khokhlova et al. (1978) indicated that cycloheximide (0.01 mg/ml) inhibited the development of chloroplast membrane system, the synthesis of chlorophyll, and the manifestation of certain photosynthetic enzymes in isolated pumpkin cotyledons. The authors concluded that there are some common acceptors of the action of the previous phytohormones. Olszewska et al. (1982), indicated that cycloheximide (2-5 µg/ml) totally inhibited protein synthesis after 2 h. application in antheridial filaments of *Chara vulgaris*. The protein synthesis was resumed after the removal of the inhibitor. Oro and Trippi (1982) followed the evolution of chlorophylls and proteins during the aging of leaf discs of *Phaseolus vulgaris*. They found that light increased chlorophyll degradation and soluble protein

content. Kinetin counteracted the light effects whereas, on the contrary, cycloheximide accelerated the light-induced chlorophyll deterioration and diminished protein content. Both kinetin and cycloheximide induced changes in membrane permeability. Kinetin suppressed the diffusion of electrolytes induced by cycloheximide. Emmerich and Radler (1983) proved that cycloheximide inhibited the induction of the synthesis of new carrier proteins in fructose fermenting *Saccharomyces bailli*, after being suspended in glucose medium. In absence of the inhibitor, the organism could induce the synthesis of new protein and became able to carry out glucose fermentation after 2 hours in the inducing glucose medium. Minamikawa et al. (1983) showed that cycloheximide added to imbibing axes of germinating *Vigna unguiculata* seeds inhibited the degradation of major globulin proteins. Such degradation of the self-sustained reserve proteins might be essential to utilize the resulting units for the synthesis of new proteins. Picton and Steer (1983), showed that in *Tradescantia* pollen tubes, reduced dictyosome activity could be detected as early as 15 min. after sowing in 1.0 µg/ml cycloheximide solution. After 30 min., vesicle production had completely ceased. These observations were discussed in relation to previous reports on the effect of cycloheximide on pollen tube growth and in relation to the synthesis and transfer of membrane proteins to secretory vesicles and the plasma membrane. Spitts-toesser and Walter (1983), indicated that reserve protein degradation

began the 2nd day of germination in cotyledons of *Cucurbita moschata* and was complete by the 6th day of germination. Removal of the embryo tissue had no effect upon the degradation rate or pattern. Cycloheximide prevented protein breakdown when applied to axisless cotyledons. Cycloheximide severely reduced protein degradation when applied on the the 3rd day of germination. Embryo tissue was apparently not required for reserve protein degradation in this species. Enzymes required for protein degradation were proved to be synthesized during germination. Cotyledon expansion was required for reserve protein degradation. The metabolic activity caused by cotyledon expansion might serve as an internal sink for the products of storage protein degradation, reducing their concentration to a non-inhibitory level. Aniol (1984) studied the induction of aluminum tolerance in wheat seedlings by low doses of aluminum in the nutrient solution. The possible role of the synthesis of the inducible Al-binding protein in the mechanism of Al tolerance was discussed. Cycloheximide completely abolished the increase in tolerance by blocking the synthesis of the binding protein. Lyubimova and Verulidze (1984) showed that in plasmalemma proteins of potato tuber discs, the incorporation of ^{14}C-leucine was completely inhibited with a decrease of temperature during the incubation and pretreatment of the discs with cycloheximide. Cycloheximide was thought to affect protein synthesis and thus indirectly modify certain cell receptor of the membrane in barley, buckwheat, and