STUDIES ON CARBOHYDRATE AND NITROGEN METABOLISM OF CERTAIN PLANT TISSUES

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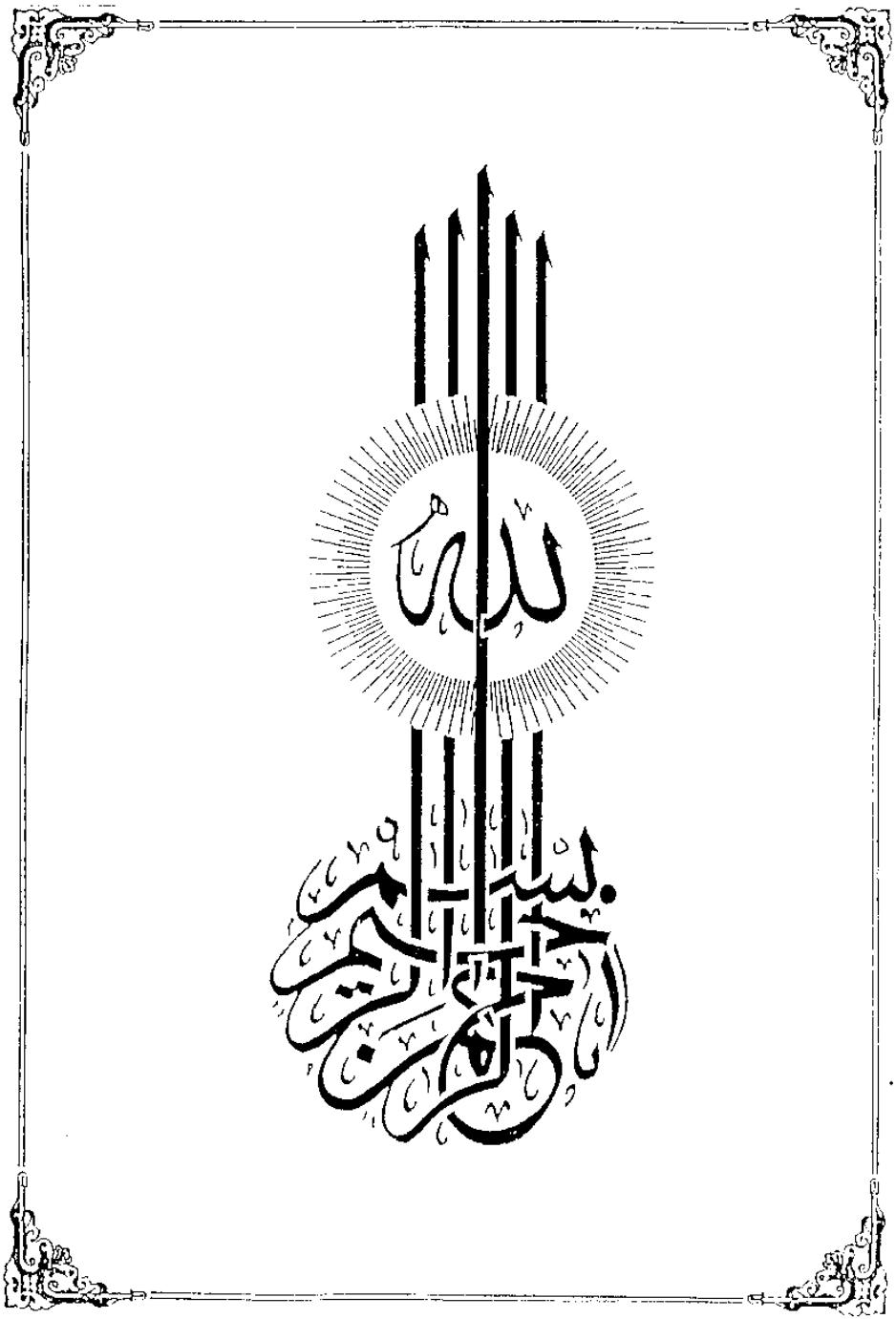
This thesis is not, to my knowledge, the same as any thesis submitted at this or at any other university.

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



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INTRODUCTION

Seed Germination

The starting process in seed germination is commonly considered to be imbibition of water. This is followed by embryo growth with the generation of sufficient force to rupture the seed coat with the consequent emergence of the radicle (Koller et al., 1962 ; Mayer and Poljakoff-Mayber, 1963).

In this study, it would be of interest to give a brief account of the chemical composition of seeds as well as the changes in breakdown and metabolism of storage products and enzyme activity during germination. The compounds known to be present in various seeds can be divided roughly into 2 groups :

(1) the normal constituents which are likely to occur in every plant tissue, and

(2) storage materials which are frequently present in seeds in large amounts.

It is estimated that lipids form the chief food reserve in the seeds of a big group of flowering plants while starch and hemicellulose form the principal reserve in the seeds of a smaller group of plants. Seeds containing proteins, which differ in chemical composition and properties from those found in other plant tissues, can belong to either group (Mayer and Poljakoff-Mayber, 1963 ; Crocker and Barton, 1953 ;

Toole et al., 1956).

In addition to carbohydrates, proteins and lipids, seeds contain minerals, tannins, phosphorus compounds, tricarboxylic acid cycle intermediates, amino acids, phenolic compounds, nucleic acids and vitamins (Mayer and Poljakoff-Mayber, 1963; Crocker and Barton, 1953; Powden, 1960). Furthermore, many species of seeds contain growth-regulating substances in variable quantities (Evenari, 1949).

Dry seed is considered as a well equipped functional unit characterized by a low rate of metabolism. Very marked changes in the rate of metabolism and in the seed composition, in its various parts, occur as soon as it is hydrated. One of the early phases of the germination process is the activation of enzymes in the region of the radicle and the increase in the respiratory rates (Toole et al., 1956). The requirement of energy for the onset of germination is satisfied by the increased rate of respiration. It has been argued that, during the early stages of seed germination, there is a grand period of respiration during which the rate of respiration rises from approximately zero to a maximum and then falls again (Stiles and Leach, 1960).

The chemical changes which occur during germination are complex in nature. They consist of :

(1) the breakdown of certain materials in the seed,

(2) the transport of materials from one part of the seed to another (especially from the endosperm to the embryo or from the cotyledons to the growing parts) and

(3) the synthesis of new materials from the breakdown products formed

During the early stages of germination of lipid seeds, a considerable quantity of the lipid content was found to disappear (Koller et al., 1962 ; Mayer and Poljakoff-Mayber, 1963 ; Moustafa, 1968). These authors also referred to the work of several investigators who showed that in seeds containing starch, a distinct fall in the latter is usually accompanied by a marked increase in the soluble sugars.

Protein is often broken down during germination with a concomitant rise in amino acids and amides followed by protein synthesis de novo in the growing parts of the embryo (Oota et al., 1956). Thus an alteration in the ratio of protein to soluble nitrogen is the first observed change during germination of lettuce seeds; an increase in soluble nitrogen being observed in germinated seeds (see Koller et al., 1962).

Amino acids may be directly utilized for the production of enzymes, growth regulators, nucleoproteins or indirectly utilised for formation of other intermediates (Dutcher et al., 1956). Soluble nitrogen may also be utilized during germination. Egami et al.(1957) showed that the small amounts of nitrates present in Vigna seeds disappear as germination

proceeds. They proved the existence of nitrate reductase system in Vigna seedling. However, there is little change in total nitrogen content of the seed during germination, although slight losses may occur especially due to leaching of nitrogenous substances.

The metabolic changes occurring in the early stages of germination are the result of the activity of various enzymes. In general, enzymes breaking down starch, proteins, hemicellulose, polyphosphates, lipids and other storage materials, rise in the activity fairly rapidly as germination proceeds (Koller et al., 1962 ; Mayer and Poljakoff-Mayber, 1963).

Use of Inhibitors in Mechanism Studies

The effect of inhibitors on plant cells has been studied extensively and their use has played a central role in the study of a wide variety of mechanisms. Unravelling of the pathways of glycolysis, organic acid oxidation and terminal electron transport is closely connected historically with studies on effects of iodoacetate, malonate and cyanide respectively (Hackett, 1960).

An ideal specific inhibitor that may act upon a single enzyme is at present unrealized. The number of substances now known as inhibitors, in suitable dosages, will react preferentially with particular groupings in enzyme molecules. Dosages and conditions may be controlled with a view to limiting their

response of a given tissue is sometimes markedly dependent on the pH of the medium. When an inhibitor is used at one pH level it acts as an inhibitor and at another as a stimulant. The extensive study of Simon and Beevers (1952) has shown closely similar pH effects with certain respiratory inhibitors which increase respiration when used at low concentration ranges.

The concentration of the inhibitor used may be critical in determining the qualitative and the quantitative nature of the response. It is not uncommon for a low concentration of an inhibitor to stimulate a reaction which is inhibited at higher concentrations. These stimulating effects are generally attributed to the inhibition of competing reactions (Hackett, 1960). However, two other mechanisms have been put forward to account for the increase in respiration rate following treatment with weak inhibitors. Thus Simon (1953) and Syrett (1958) suggested uncoupling of oxidative phosphorylation to explain the increased respiration; while phosphorylation is inhibited there may be no measurable effect on the O_2 uptake or it may be stimulated due to an increase in the supply of the phosphate acceptors. The second possibility is that the increased respiration may be due to increased accessibility of substrates to enzymes (Barker and Mapson, 1964 ; Barker and Younis, 1965b ; Younis, 1969 ; Younis et al., 1969a).

Without giving evidence, Boswell (1950) stated that the

substrates which inhibit respiratory activity can be divided into 2 groups. The first group contains malachite green and iodoacetate which increase cell permeability. The second group contains KCN, NaF and NaN_3 which are without effect upon the permeability in concentration which markedly reduce the rate of respiration. In the light of his results with Brassica napus he suggested that the control of cell permeability is located in a particular part of the respiratory mechanism and only when that particular system is inhibited does increased cell permeability develop with loss of cell contents.

Lundegardh and Stenlid (1944) considered the excretion of nucleotides and flavonones from living roots to be the result of simple leakage of substances from the cells. Generally speaking, it is often difficult to say to what extent an excretion is connected with metabolism or not. This applies especially to the results obtained in experiments where the effects of inhibitors are studied (Helder, 1956).

Previous Work With Inhibitors

The literature is full of articles dealing with inhibitions and stimulations of respiration of plant tissues using different concentrations of respiratory inhibitors (see James, 1953 ; Hackett, 1960). These changes in the rate of respiration of plant tissues were always accompanied by changes in the metabolism of the cell and in the permeability of cell membranes.

There is no attempt in this thesis to survey the voluminous reports dealing with the effects of respiratory inhibitors on plant tissues. We are rather going to give a brief account of, and bring into focus, some of the work with the respiratory inhibitors used in the present study.

a- Previous work with iodoacetate.

Iodoacetate was first introduced by Lundsgaard in 1930 as an inhibitor of muscle glycolysis. The satisfactory application of iodoacetate to higher plant tissues was first reported by Turner (1938) who stated that iodoacetate has essentially the same effect on fermentation and respiration but acts more slowly on respiration than on fermentation for any given external concentration. Since that work was published, many investigators have carried on work on iodoacetate in order to study the inhibitory action and to investigate possible alternative pathways of carbohydrate metabolism.

The respiration of spinach leaves was investigated by measuring gaseous exchanges of excised leaf sections and by an examination of individual enzymes and enzyme systems in vitro (Bonner and Wildman, 1946). Iodoacetate inhibited the respiration of spinach leaves and they stated that it is so nonspecific in its action that no great stress can be laid on its activity, in the present case, in relation to any particular enzyme. Laties (1949) showed that the extent to which barley root respiration was inhibited by iodoacetate was related to

pH. At pH 5.0, 75% inhibition was obtained whereas at pH 6.5 no inhibition was apparent and that led him to state that the pH affects the penetration of the inhibitor rather than its action within the cell.

Earlier investigators of iodoacetate poisoning suggested that a phosphorylation process was primarily inhibited (Beevers, 1950). Working with zymin preparation from yeast he observed that when iodoacetate was applied in concentrations greater than 10^{-3}M fermentation of either glucose or hexosediphosphate was arrested almost completely. He explained his results on the supposition that some enzyme system in the zymase complex is susceptible to iodoacetate. Using iodoacetate, James et al. (1944) have shown that it inhibited oxidation of triosephosphates; these esters accumulating in treated barley seedlings. Christiansen et al. (1949) studied the effect of iodoacetate upon pea hypocotyl pieces. They found that iodoacetate, at the same time, inhibited respiration and caused a more rapid decrease in sugar content compared to the controls.

Iodoacetate (0.33×10^{-3}M and 0.165×10^{-3}M) increased the CO_2 output and the inorganic phosphate content of ivy leaves while no significant change in sugar content was observed (Contreiras, 1945). He postulated that iodoacetate promotes the hydrolysis of a high-energy-content phosphate compound, thus increasing the respiration but diminishing the energy available for certain cell processes.