Summary

Light and O_2 are essential for a rapid bleaching of the pigment system of broadleaf bean by paraquat (1,1-dimethyl-4,4-dipyridylium dichloride). This bleaching does not appear to be directly related to physiological activity but to the destruction of a protective system which normally prevents photo-oxidation. Light, but not O_2, is also essential for the changes in membrane permeability brought about by paraquat in mesquite (*Prosopis glandulosa*), honeysuckle (*Lonicera saponica*), and broadleaf bean (*Phaseolus vulgaris*). Changes in permeability are also temperature dependent. Light is not essential for paraquat's effect on root elongation in mesquite.

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Changes in Composition During Development and Maturation of Maize Seeds[1, 2]

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Introduction

The analysis of metabolites during germination has shown that the endosperm of the mature corn grain contains very little RNA (10). Since large amounts of protein, particularly the storage protein, zein, are synthesized in the endosperm (2), and RNA is intimately involved in protein synthesis (1), such a low level of RNA in the endosperm is unexpected. Studies on the development of wheat grains have shown that during the period of rapid increase in endospermal protein, there is a related increase in RNA content (4). Furthermore, it has been suggested that the storage proteins are formed on the ribosomes of the endoplasmic reticulum in the endosperm and afterwards secreted internally to form the protein storage bodies (4). Similar protein storage bodies have been described in the endosperm of corn seeds (3). These considerations suggest that RNA synthesis must occur during the development of the endosperm of the corn grain. This RNA could be degraded during the maturation of the endosperm since it has been shown that the mature corn endosperm contains a high level of ribonuclease activity (8). The results of Matsushita (14) on the development of rice and wheat seeds indicate that RNA synthesis occurs very early in the developmental sequence, and that the RNA may then be degraded.

Minimal information is available concerning the changes in metabolites which occur during the development and maturation of the corn seed. Consequently an investigation was undertaken to determine the changes in dry weight, water, nitrogen fractions, fat, RNA, DNA, soluble nucleotides, soluble sugars and ribonuclease activity occurring in the endosperm and embryo of the developing seed.

Materials and Methods

Two inbred (WF9 and M14) lines of corn (*Zea mays*) were planted in field plots at the Experiment Station Farm at Urbana, Illinois, in the spring of 1962. Sixty plants each of M14 and WF9 were self-

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pollinated on July 30 and August 3, respectively. An initial sample of the grain was taken the day following pollination, and 10 samples were taken on every third day through to September 17. The remaining ears were harvested on October 18 and stored at room temperatures. Additional samples of the harvested ears were taken in December.

At each sampling, 3 ears were randomly selected from each inbred plot and transported directly to the laboratory. Seeds were removed from the central region of each ear, mixed, and used for the experimental determinations. For the first 13 days after pollination the whole grain, which at this stage consisted largely of testa-pericarp, was analyzed. Duplicate lots of 30 to 50 seeds were used for each of the determinations, which were carried out as previously described (9, 10).

Embryos were dissected from the seed sampled on the sixteenth day after pollination, and from all subsequent samples. The embryo (scutellum plus embryo axis) and the remainder of the seed, which by this stage in the development was largely endosperm, were analyzed separately. Duplicate lots of 20 endosperms and 50 embryos were used.

Experimental Results

The changes in dry weight, water and metabolite content and ribonuclease activities of the developing and mature corn seed are presented in figures 1 through 12. Since the results obtained with the WF9 and M14 inbreds were very similar, showing only small quantitative differences, the WF9 data only will be presented. Comparable analytical data for the mature seed, harvested in October and stored at room temperature until December, are included as a measure of the fully-developed grain (seeds were soaked for 4 hours in water to permit dissection).

Discussion

Cell division of the endosperm is essentially complete within 28 days following pollination. At this stage the endosperm has maximum DNA content, indicating completion of cell division. RNA content has also reached a maximum value, and the initial rapid phase of protein synthesis has leveled off. The parallel increases in protein, RNA, and DNA during this period are typical of an actively growing tissue, and are similar to those reported during the development of wheat grain (4). This initial phase of development of the endosperm is also characterized by a rapid accumulation of soluble constituents, presumably via translocation from the plant. The water content of the endosperm is maximal at this stage. Sugar content increased rapidly, reaching a maximum content within 20 days from pollination, whereas soluble nitrogen, amino acid and soluble nucleotide contents peak around 28 days.

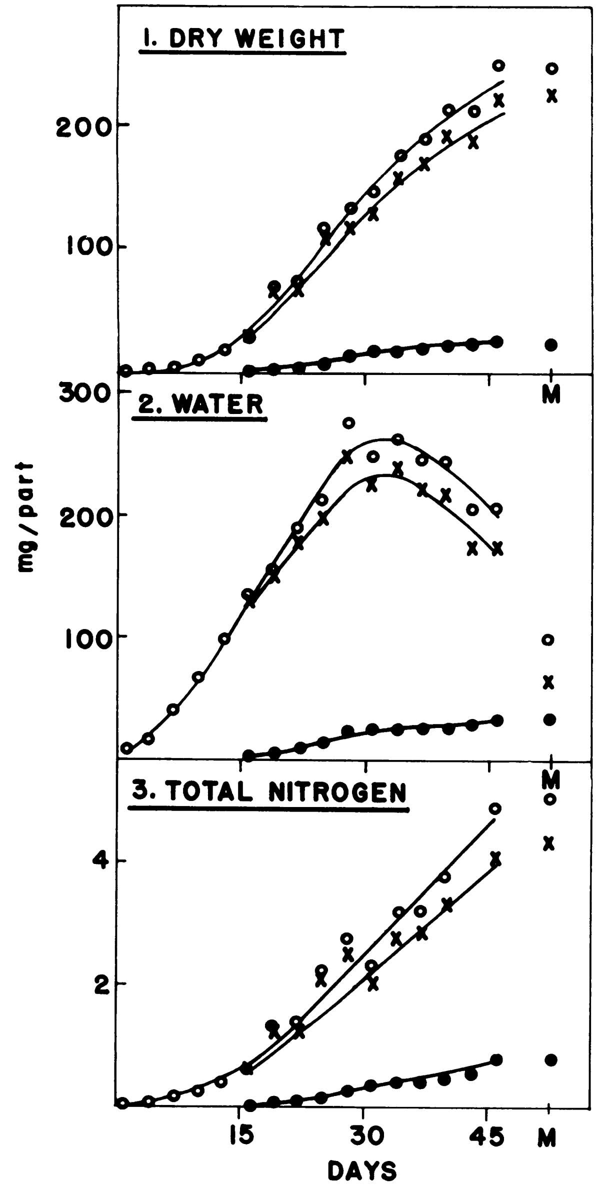

FIG. 1–3. Changes in dry weight, water and total nitrogen of the embryo and endosperm over a 46-day developmental period after pollination. M: comparable data from the analysis of mature grains (see Methods). O-----O, whole grains; X-----X, endosperm; ●-----●, embryo.

The second increase in protein content of the endosperm, from about 40 days, is indicative of the production of specialized storage protein. It has been generally recognized that different proteins are synthesized at different stages in development, and in particular, that reserve proteins are synthesized during the later stages (17, 18). This concept is supported by the similarity of protein formed in late stages of seed development to that found in protein storage bodies, with respect to amino acid compo-

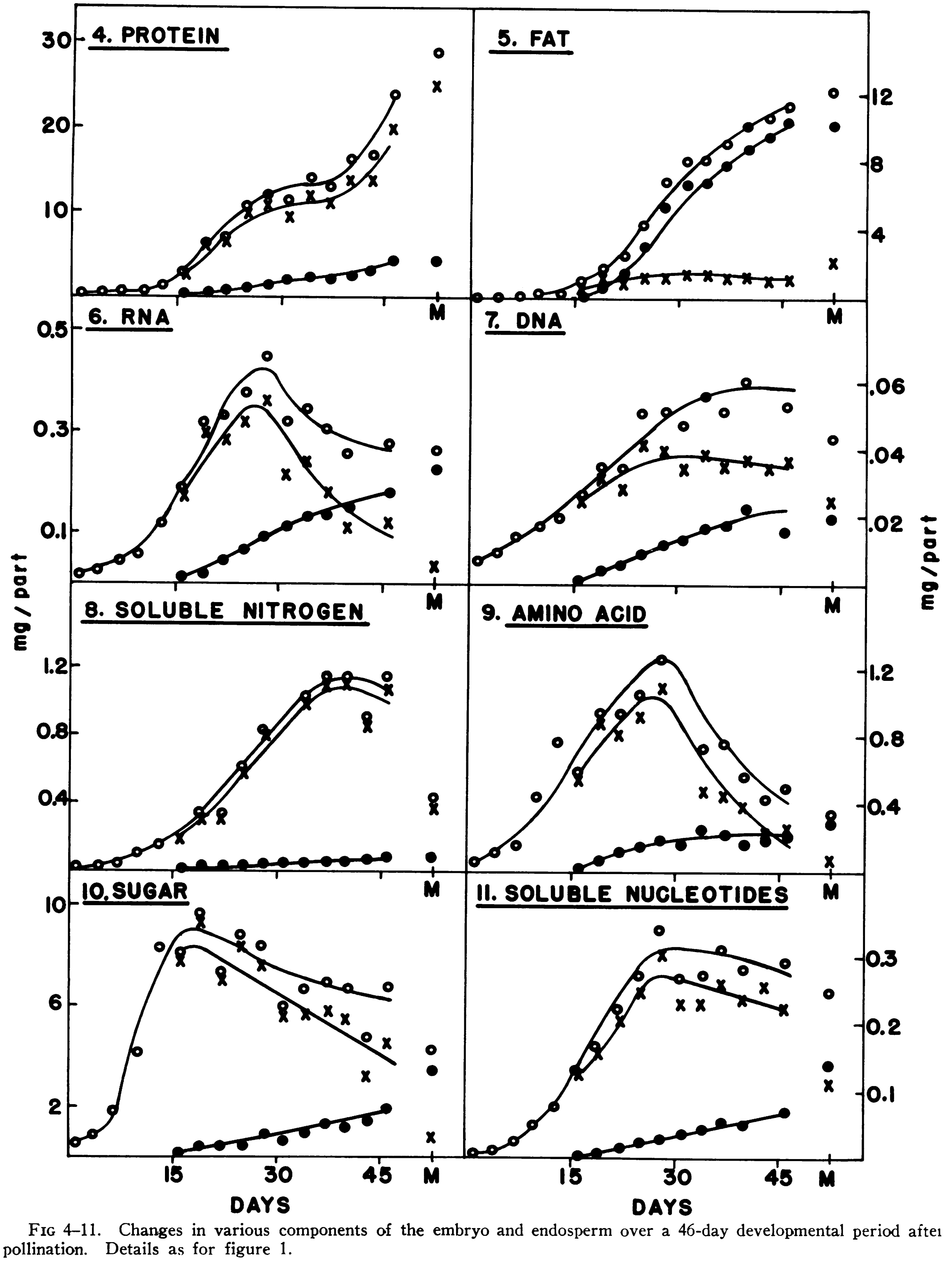

FIG 4–11. Changes in various components of the embryo and endosperm over a 46-day developmental period aftei pollination. Details as for figure 1.

FIG. 12

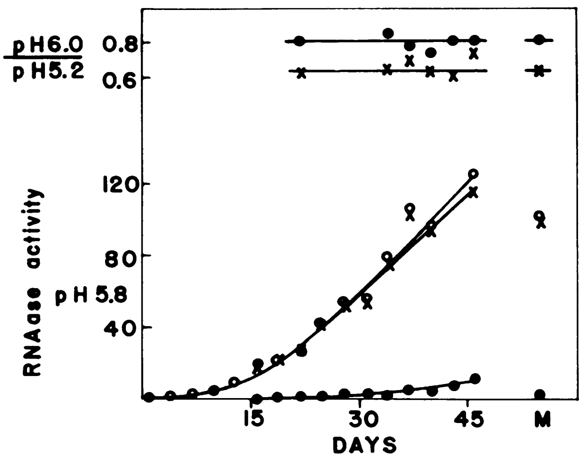

FIG. 12. Changes in ribonuclease activity (pH 5.8) in the embryo and endosperm over a 46-day developmental period after pollination. Included is the ratio of activity at pH 6.0 to pH 5.2. Details as for figure 1.

sition (11, 12). The decrease in soluble nitrogen and amino acid content of the endosperm after 28 days suggests that these components are utilized for the production of the storage protein, in this case zein. The almost linear increase of dry weight of the endosperm over this period (from 30–46 days) and the decrease in soluble sugar content, suggests that a considerable amount of starch was also being synthesized. Although the DNA content of the endosperm remained constant after the maximum value had been reached at 25 to 28 days, endospermal-RNA decreased very rapidly during this period to about one-third of the maximum value. This decrease in RNA was associated with a rapid increase of endospermal ribonuclease activity, suggesting, but not proving, a casual relationship. The ratio of ribonuclease activities at pH 6.0 and pH 5.2 was 0.64. Since this value is close to the ratio value 0.60 obtained with pure ribonuclease A (10), the ribonuclease activity of the endosperm must essentially be of the A type. Previous studies of germinating corn endosperm are consistent with this view (10).

Many of the metabolic processes had not reached completion by the end of the 46-day experimental period, as shown by a comparison of the 46-day analysis with that of grain which had matured for an additional month on the plant, and then was stored for 2 months after harvesting. The increase in endospermal protein which occurred during this time was considerably larger than the increase in total nitrogen, but could be accounted for by decreases in the soluble nitrogen and amino acid content. The nucleic acid content also changed during the final maturation, with both RNA and DNA decreasing in the endosperm. The levels of all the soluble constituents, soluble nitrogen, amino acids, sugar, and nucleotides, were much lower in the fully matured endosperm than at the end of the experimental period.

Development of the embryo proceeded in an essentially linear manner during the 46-day period, and at the end of the time was complete in terms of the dry weight, water, total nitrogen, protein, fat, DNA, soluble nitrogen and amino acid content. The further increases in RNA, sugar and soluble nucleotides which occurred during the final maturation could result from the movement of these constituents from the endosperm into the embryo. A slight, but consistent, increase in ribonuclease activity of the embryo was noted over the 46-day period. During this period, there was no change in the pH 6.0 to pH 5.2 ratio value (0.82) thus showing that ribonuclease A and ribonuclease B activities were increasing at comparable rates, a condition which was also characteristic of the scutellum during germination (10).

The endosperm of the mature corn grain contains very little reserve RNA, and consequently, on germination, the increase in nucleic acid during the initial stage of embryo growth is completely due to de novo synthesis (8). This contrasts with other cereal grains, where utilization of storage nucleic acid could account for part of the increase in embryo-nucleic acid during this initial germination phase (7, 13, 15, 16). The pattern of development of the corn grain suggests, however, that this difference between corn and the other cereals may result from the relatively greater development of the corn embryo prior to germination. The loss of RNA from the endosperm during the later stages of development could account for the increase of embryo RNA during this period. The developmental pattern, together with the high stability of ribonuclease A (10), also readily explains the existence of the high ratio of ribonuclease to RNA found in the endosperm of the mature corn grain.

The components that were measured account for approximately 20 % of the total dry weight. From the work of Earley (5) and Evans (6), it may be assumed that 60 to 70 % of the total dry weight would be starch and dextrins. Cellulose, waxes, minerals, etc. would account for the remainder of the total dry weight.

Summary

The changes of weight and various chemical constituents, nitrogen fractions, RNA, DNA, fat, soluble sugar and nucleotides, have been determined over a 46-day period after pollination in the endosperm and embryo of developing corn grains. The development of the endosperm has been considered in 2 phases. The initial phase, lasting for the first 28 days from pollination, was characterized by an accumulation of soluble constituents (soluble nitrogen, amino acids, sugars and nucleotides) and by the synthesis of protein, RNA and DNA. During the second phase, from 28 to 46 days, there was a utilization of the

soluble constituents, with further increases in protein content. Although DNA content stayed constant, the RNA content decreased and was accompanied by an increase in ribonuclease activity. Further changes occurred during the final maturation of the grain that included an increase in protein and decreases in the nucleic acids and soluble constituents. The embryo developed in a linear manner during the 46 days, and at this stage the development of the embryo was essentially complete. The pattern of endosperm development and the stability of ribonuclease accounted for the high ratio of ribonuclease to RNA observed in this organ during the germination of the mature grain.

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