Study on the Fatty Acid Composition During Germination of Peanuts Treated With Growth Regulators

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Abstract

The fatty acid metabolism during germination of a special variety of peanuts treated with growth regulators, viz., GÅ, AA, Suc, Sul, MH and DW have been studied in sterile media for 1, 3, 6, 10, 14 and 18 days. No marked variation in the oil content of the control and the treated seeds is found during the initial period of germination. Initial growth is explained on the basis of energy supplied by the FFA and carbohydrates (as they are found decreasing during the 24 hr of germination). Practically no accumulation of FFA is observed throughout the complete period of germination. The mode of utilization of neutral oil with the period of The germination is correlated with growth. varied effect of growth regulators on the fat metabolism is explained assuming their various effects on mitochondria, the enzyme center, and on the process of photosynthesis. Active metabolism is marked from the sixth day of germination for both treated and control seeds. From the beginning of germination, interconversion of fatty acids is marked. Metabolism of fatty acids during germination is reported with the preferential utilization of 18:2 acid.

Introduction

P_{RESOWING CHEMICAL TREATMENTS are today receiving considerable attention in attempts to understand and explain the various aspects of plant physiology and thereby the related properties of the plant.}

Kurnik et al. (1) exposed soybean to various photoperiods. At each photoperiod gibberellic acid (GA) was sprayed on the plants. The treatment was reported to increase the oil content of soybean. De Leo (2) marked the effects of indolebutyric acid (IBA), GA and eosin by applying presowing treatments to Arachis hypogaea. After germination the plants were alternately sprayed with these solutions for 80 days. The treatment with IBA resulted in crop increase with higher protein content and lower oil content while treatment with GA resulted in the reverse with respect to crop yield, protein and oil contents. Kaul and Kapoor (3) studied the effect of different concentrations of GA on the physiological aspects and volatile oil content of Anethum graveolens and A. sowa seeds. The volatile oil in the seed was found to increase up to 50 p.c. in A. graveolens and about 30 p.c. in A. sowa. Other investigators (4,5) have also reported several physiological changes along with changes in the volatile oil yield in a variety of seed plants treated with GA. Ogzewalla (6) studied the effect of aqueous solution of GA on sesame, castor bean, sunflower and flax plants with respect to changes in the chemical constituents of the essential oil. The higher saponification value of oil, indicating shorter chain fatty acids, was marked. Changes were also reported in the quality of oils from plants that had insignificant or no visible morphological changes.

Lambou et al. (7) observed that maleic hydrazide (MH) could contribute to inhibition of free fatty acid formation in the seeds during field exposure and during storage previous to sampling.

Thus, most of the work reported in the literature, on the effect of growth regulators, is confined either to the physiological role played by such treatments or to the yield of essential oils or crops. Attempts are not made to explain the role played by the growth regulators on the utilization of fat.

Hence the present investigation has been undertaken to study the effect of growth regulators on fat utilization and fatty acid composition during germination.

Experimental Procedures

As described in previous work (8), 4 sets of germinating seedlings, not less than 10 from each treatment, were removed from sand at the end of specified periods of germination, cleaned with cold distilled water and dried. Extraction of oil from dry seedlings was carried out according to the procedure used by Patel et al. (9). Separation of FFA and glycerides was carried out by silicic acid column chromatography (10,11). The neutral oil was subjected to silica gel column chromatography according to the technique of Quinlin and Weiser (12), for the separation and determination of mono-, di-, and triglycerides in neutral oil: The method used for the preparation of methyl esters of all the samples as mentioned above is essentially the same as that used by Stoffel et al. (13).

The methyl esters obtained were analyzed individually with the Beckman GC-2/gas chromatograph at 206 C, using hydrogen as a carrier gas at 30 psig. A 12 ft, $\frac{3}{16}$ in. stainless steel column containing 30 p.c. diethylene glycol succinate (DEGS) on 40–60 mesh acid washed chromosorb-W was used. The current strength of 300 ma was fed to the filament of the thermal conductivity detector cell. The difference in the thermal conductivity was indicated on a recorder having sensitivity of 1 mv full scale deflection. For correct identification of the component acids in each sample, a standard (Hormel) mixture of methyl esters was run through the same conditioned column under This enabled the identical conditions. correct identification of the fatty acid esters of the test samples. Percentage composition of each fraction was evaluated from the peak area of individual components, measured by mechanical integrator.

Results

The rate of utilization of neutral oil in each treatment is expressed on 100 g seedlings and on the original weight of the seeds (Table I). It is observed that presowing chemical treatments are effective on the mode of utilization of the neutral oil after the third day. The percentage of FFA, calculated on the original weight of the seeds (Table I). It is observed is a reduction during the early stages of germination.

TABLE I Effect of Growth Regulators on Neutral Oil and Free Fatty Acids of Peanuts During Germination

| | | | | | | | | | | Tre | atment | s ^a | | | | | | | | | |
|------------------|---------|------|-----|------|------|-----|-------------|------|-----|------|--------|----------------|-------------|-------------|-----|-------------|------|-----|-------------|-------------|--------------|
| Germina- tion | <u></u> | GA | | | AA | | | SUC | | | MH | | | SUL | | | DW | | cc | NTRO | L |
| days | A | в | C | A | В | C | A | В | C | A | в | C | A | в | C | A | в | С | A | В | C |
| 0 | 48.8 | 48.8 | 0.6 | 48.9 | 48.9 | 0.5 | 49.1 | 49.1 | 0.4 | 49.1 | 49.1 | 0.4 | 48.6 | 48.6 | 0.3 | 48.5 | 48.5 | 0.4 | 48.9 | 48.9 | 0.4 |
| ĭ | 48.7 | 48.5 | 0.3 | 48.8 | 48.7 | 0.2 | 48.8 | 48.5 | 0.2 | 48.9 | 48.7 | 0.2 | 48.5 | 48.4 | 0.1 | 48.4 | 48.3 | 0.1 | 48.6 | 48.8 | 0.2 |
| 3 | 48.0 | 47.5 | 0.1 | 47.4 | 47.1 | 0.2 | 47.3 | 46.8 | 0.1 | 48.5 | 47.9 | 0.2 | 48.2 | 48.0 | 0.1 | 47.9 | 47.4 | 0.3 | 48.1 | 47.6 | 0.3 |
| 6 | 42.7 | 41.5 | 0.4 | 41.9 | 40.6 | 0.3 | 42.9 | 40.8 | 0.3 | 43.0 | 42.1 | 0.5 | 43.3 | 42.4 | 0.3 | 43.5 | 43.0 | 0.3 | 33.1 | 32.5 | 0.4 |
| 10 | 30.7 | 30.1 | 0.6 | 29.9 | 29.2 | 0.5 | 31.1 | 30.2 | 0.7 | 28.1 | 27.6 | 0.9 | 33.2 | 32.8 | 1.0 | 30.1 | 29.4 | 0.7 | 18.7 | 17.9 | 0.4 |
| 14 | 26.5 | 24.2 | 0.7 | 20.5 | 18.1 | 0.4 | 27.8 | 25.3 | 0.7 | 26.3 | 22.5 | 0.3 | 31.5 | 30.9 | 0.2 | 25.7 | 24.3 | 0.2 | 13.4 | 12.1 | 0.1 |
| 18 | 23.3 | 22.9 | 0.9 | 18.9 | 17.0 | 0.5 | 26.8 | 24.3 | 0.5 | 21.0 | 20.2 | 0.9 | 28.4 | 27.5 | 0.3 | 24.0 | 23.1 | 0.2 | 7.8 | 6.1 | \mathbf{T} |

Gibberellic acid (GA) 30 mg/100 ml. Ascorbic acid (AA) 15 mg/100 ml. Sucrose (SUC) 6 g/100 ml. Maleic Hydrazide (MH) 3×10^{-4} M. Sulphanilamide (SUL) 1×10^{-2} M. Glass distilled water (DW). A: % neutral oil in seedlings. B: % neutral oil calculated on original wt of seeds. C: FFA in 100 g seeds. T: traces.

C: FIA T: traces.

With increase in the period of germination the FFA seem to vary slightly in each treatment.

In order to have a clear picture of the utilization of component acids, the composition of the neutral oil determined from GLC data and calculated on the original weight seeds, is presented in Table II. The GLC data indicate that the neutral oil in the present variety of peanuts consists of 16:0, 18:0, 18:1 and 20:0 as the component fatty acids, following the notation of Farquhar et al. (14). As shown in Table II there is little variation in the amount of 16:0 acid up to the third day of germination. This is true for the control as well as for the treated seeds. The 16:0 acid is metabolized rapidly after the third day of germination. The amount of 18:1 acid is found to decrease with a corresponding increase in the amount of 18:2 acid up to the third day of germination of the control seeds. The same pattern is followed in the treated seeds. On the sixth day of germination of the control seeds, the amount of 18:1 and 18:2 acids is found to decrease, the decrease in 18:2 being rapid. In case of treated seeds, decrease in 18:2 is observed, but the amount of 18:1 acid is almost the same on the sixth day of germination. In the case of control and in cases treated with regulators, both the 18:1 and the 18:2 acids are found to decrease from 10 days onwards.

Discussion

It is observed from Table I that there is no great variation in the neutral oil content of the seedlings of the control nor of treated seeds up to the third day of germination. Significant variation in the neutral oil contents begins after the third day of germination in all treated and control seeds. The starch, total sugar (15) and FFA (Table I) of the control and the treated seeds are found to decrease on the first day of germination. One can conclude that in the initial stage of germination, the energy required by the developing radical is obtained only from the metabolism of carbohydrates and FFA. Oil is metabolized at a later stage.

Up to the sixth day of germination the rate of neutral oil metabolism is found almost the same for

TABLE II

| Composition | of | the | Fatty | Acids | \mathbf{in} | Neutral | Oil | Calculated | \mathbf{on} | Original | Weight | of | Seeds | (100 | g). | |
|-------------|----|-----|-------|-------|---------------|---------|-----|------------|---------------|----------|--------|----|-------|------|-----|--|
| - | | | | | | | | | | | | | | | | |

| Germina- tion days | Acids | GA | AA | SUC | МН | SUL | DW | Control |
|--------------------------|--------------------------------------|-------------------------------------|-------------------------------------|--|--|--|--|--|
| 0 | 16:0 18:0 18:1 18:2 20:0 | $7.9 \\ 1.1 \\ 22.4 \\ 16.8 \\ 0.7$ | $8.1 \\ 1.1 \\ 22.5 \\ 16.6 \\ 0.6$ | $7.9 \\ 1.1 \\ 21.7 \\ 17.7 \\ 0.7$ | $8.1 \\ 1.2 \\ 22.0 \\ 17.2 \\ 0.6$ | 8.0 1.3 21.8 17.0 0.5 | $\begin{array}{c} 8.1 \\ 1.4 \\ 21.8 \\ 16.8 \\ 0.5 \end{array}$ | 7.8 1.3 22.2 17.0 0.6 |
| 1 | 16:0 18:0 18:1 18:2 20:0 | $7.1 \\ 0.5 \\ 21.1 \\ 19.1 \\ 0.7$ | $7.5 \\ 0.7 \\ 21.6 \\ 18.5 \\ 0.4$ | 7.4 0.6 20.9 19.2 0.5 | $7.7 \\ 0.6 \\ 21.0 \\ 18.9 \\ 0.4$ | $7.5 \\ 0.7 \\ 21.1 \\ 18.5 \\ 0.6$ | $7.2 \\ 0.6 \\ 21.0 \\ 18.8 \\ 0.7$ | 7.5 1.3 21.0 19.0 0.6 |
| 3 | 16:0 18:0 18:1 18:2 20:0 | $7.0 \\ 0.2 \\ 18.7 \\ 21.2 \\ 0.5$ | $7.0 \\ 0.2 \\ 18.7 \\ 20.5 \\ 0.6$ | 6.9 0.3 18.2 20.8 0.6 | $\begin{array}{c} 6.8 \\ 0.2 \\ 17.8 \\ 22.8 \\ 0.4 \end{array}$ | $\begin{array}{c} 6.8 \\ 0.4 \\ 18.8 \\ 21.7 \\ 0.3 \end{array}$ | $\begin{array}{c} 6.9 \\ 0.3 \\ 19.1 \\ 20.5 \\ 0.5 \end{array}$ | $\begin{array}{c} {\bf 6.9} \\ {\bf 0.2} \\ {\bf 19.1} \\ {\bf 20.8} \\ {\bf 0.6} \end{array}$ |
| 6 | 16:0 18:0 18:1 18:2 20:0 | $5.6 \\ 0.5 \\ 19.4 \\ 15.5 \\ 0.4$ | $5.6 \\ 0.6 \\ 19.0 \\ 15.1 \\ 0.3$ | $5.4 \\ 0.8 \\ 18.5 \\ 15.7 \\ 0.3$ | $5.9 \\ 0.6 \\ 19.2 \\ 16.3 \\ 0.2$ | 5.7 1.0 18.6 16.9 0.3 | $5.9 \\ 0.9 \\ 18.9 \\ 16.9 \\ 0.4$ | $\begin{array}{r} 4.3 \\ 0.7 \\ 14.5 \\ 12.6 \\ 0.5 \end{array}$ |
| 10 | 16:0 18:0 18:1 18:2 20:0 | 4.1 0.9 14.6 10.3 0.3 | $3.9 \\ 1.1 \\ 13.8 \\ 10.2 \\ 0.3$ | $\begin{array}{r} 4.2 \\ 0.8 \\ 13.8 \\ 11.1 \\ 0.3 \end{array}$ | 3.8 1.0 13.3 9.2 0.3 | $\begin{array}{r} 4.4 \\ 0.9 \\ 14.8 \\ 12.1 \\ 0.6 \end{array}$ | $\begin{array}{r} 4.1 \\ 0.9 \\ 13.3 \\ 10.8 \\ 0.2 \end{array}$ | 2.5 0.5 8.2 6.6 0.1 |
| 14 | 16:0 18:0 18:1 18:2 20:0 | $3.4 \\ 0.2 \\ 10.9 \\ 9.4 \\ 0.3$ | $2.5 \\ 0.2 \\ 8.1 \\ 7.0 \\ 0.2$ | 3.4 0.2 11.5 9.9 0.2 | 3.2 0.2 9.8 9.0 0.3 | $\begin{array}{r} 4.3 \\ 0.4 \\ 13.7 \\ 12.1 \\ 0.4 \end{array}$ | 3.4 0.3 11.1 9.2 0.3 | 1.7 0.1 5.4 4.7 0.1 |
| 18 | 16:0 18:0 18:1 18:2 20:0 | 3.1 0.3 10.2 8.9 0.4 | 2.4 0.2 7.7 6.4 0.3 | 3.5 0.4 10.7 9.2 0.4 | 2.8 0.2 9.3 7.7 0.3 | 3.9 0.5 12.2 10.6 0.3 | 3.3 0.4 10.3 8.8 0.3 | 0.8 0.1 2.8 2.3 0.1 |

all the treated seeds. After this period it is different for different treatments. It is less in Sul treated seeds in comparison to the other treated seeds. For Suc, DW and GA treated seeds it is practically constant but is greater than that of Sul treated ones. This in turn was found to increase in MH and AA treated seeds in succession (Table I).

When neutral oil metabolism is viewed in terms of growth and metabolic products, it was observed that up to 12 days of germination, plant heights of the control, GA and AA treated seeds on germination did not vary to an appreciable extent. The plant heights of MH and DW treated seeds had similar increase throughout the germination period and were lower than that of control, GA and AA treated seeds. Still lower height was observed in the plants of the seeds treated with Suc and Sul in sequence (16). In the initial stages of germination metabolic processes are slow and the growth is mainly ascribed to the energy supplied from free sugars, starch and FFA. Variation in growth due to treatments may be ascribed to the variable effects on mitochondria, and thereby to the specific enzymes required for the interconversion and utilization of free sugars, starch and FFA. No appreciable change in neutral oil may be attributed to these causes. However, as germination proceeds, because of higher metabolic state, cell division sets in and the growth, as well as the metabolism of neutral oil, is affected by each treatment. In Sul treated seeds the growth is inhibited to a maximum extent, with accompanying lower utilization of neutral oil. Suc acts as an inhibitor of the growth on account of its high concentration (17). The growth in these seedlings is little more than that observed in Sul treated seeds. Therefore the neutral oil metabolism in Suc treated seeds is slightly increased during germination.

GA, DW and Suc treated seeds are found to have practically the same rate of neutral oil metabolism on germination, even though there is difference in growth. This may be due to the deactivating effect of DW treatment prior to germination on certain enzymic activities, resulting in the decrease of growth compared to the control seedlings and hence the utilization of neutral oil. Even though GA acts as a growth promoter, as is really the case in the present work, the rate of neutral oil metabolism is less than that of MH treated seeds. Kozlova and Ermolaeva (18) have mentioned that MH promotes a more rapid formation of tissues and accumulation of starch in leaves. GA is reported to promote maltase activity (19) which in turn hydrolyzes the raw starch to glucose. The action of GA on seedling growth may be in part due to stimulation of maltase activity. Zakhar'yants and Ionesova's (20) experiments show that the treatment of cotton plant leaves with GA results in increased photosynthesis. The starch content is found to increase on germination in the MH treated seeds (15). This may promote formation of tissues in absence of suitable activities to utilize it for promoting the growth. On account of tissue formation, photosynthesis may be affected and the energy supplied to growing seedlings may be derived from the metabolism of the neutral oil only. In the case of GA neither starch nor total soluble sugars are found to accumulate, indicating their utilization by growing plants. Further these seedlings may derive a part of their energy through photosynthesis. Thus in GA treated seeds the growth is dependent not only on the energy derived from the neutral oil metabolism but also on other processes which stimulate the growth. Hence, even though the growth of GA seedlings is high, the fat utilization is reduced.

It is observed that after 10 days of germination the growth in AA treated seeds is less in comparison to GA treated ones. Further, the starch content in AA treated seeds is found to increase and reach the value of MH treated seeds beyond 10 days of germination. Although AA has stimulatory effect on the growth of the plants, accumulation of the starch content in the growing plants may result in a decrease of photosynthesis and eventually in an increase of fat metabolism. Thus the effect of treatments is an indirect one and becomes pronounced as germination progresses. It is concluded that growth is not the only factor responsible for the consumption of fat.

Present work on the metabolism of neutral oil and the growth of plants with different treatments indicates that the utilization of neutral oil starts in an appreciable amount from the sixth day of germination. The maximum lipase activity attained in various treatments on the sixth day or thereafter (16) supports the above conclusion.

The period of intense metabolism is between the 6th and the 18th day of germination for all the seeds. During this period different metabolic products affecting metabolism are found to have changed considerably. Considerable decrease of the neutral oil in the present investigation may be ascribed to its conversion into carbohydrates, free amino acids and related materials necessary for the respiration and seedling growth (16).

A slight decrease of FFA (Table I) during the first 24 hr of germination may be ascribed to their utilization by growing radical. The data also show that their amount in 100 g of seeds for any treatment and for any period of germination remains practically constant. In view of the expected hydrolysis of fat during germination, due to the presence of large amounts of water absorbed by the seeds, there must be an increase in FFA rather than remaining practically constant. Hence two possibilities arise: there is no hydrolysis of fat during germination or whatever acids are produced due to hydrolysis are metabolized. But as it is observed that the percentage composition of FFA changes (16), even during the early stage of germination, one is inclined to conclude that there is a preferential metabolism of some of the acids which are already present or which might have been produced either by hydrolysis or lipolysis. Furthermore, the considerable decrease of the neutral oil after the sixth day of germination does not result in appreciable change of FFA in these seeds. This can occur only when the acids liberated from the triglycerides are metabolized to completion.

Normally the percentage composition of fatty acids is reported during germination and from these results, the mode of utilization of the component acids is interpreted. The results, expressed as percentage fatty acid composition of neutral oil during germination, may not necessarily give the correct picture regarding the rate of metabolism of the component fatty acids. Mention may be made that if a particular fatty acid is not metabolized at all but others are metabolized, the percentage fatty acid composition will indicate increase in the percentage of that particular acid. Thus it will apparently appear that acid has been synthesized. In order to have a clear picture regarding the mode of utilization of component fatty acids, the results are reconsidered on the basis of their

| Name of | | | | Gern | ination | days | | |
|----------------|------------------------|--|-------------------|-------------------|-------------------|------------------|------------------|---|
| treat- ment | Acids | 0 | 1 | 3 | 6 | 10 | 14 | 18 |
| GA | 16:0 18:1 18:2 | 100 100 100 | 90 94 114 | 89 83 126 | 71 87 92 | $51 \\ 65 \\ 61$ | 43 49 56 | 39 46 53 |
| AA | 16:0 18:1 18:2 | $100 \\ 100 \\ 100$ | 93 96 111 | 86 83 123 | 69 84 91 | 48 61 61 | $31 \\ 36 \\ 42$ | 8(34 39 |
| Suc | 16:0 18:1 18:2 | 100 100 100 | 94 96 108 | 87 84 118 | 68 85 89 | 53 64 63 | 43 53 56 | 44 49 48 |
| мн | $16:0 \\ 18:1 \\ 18:2$ | $100 \\ 100 \\ 100 \\ 100$ | 95 95 109 | 84 91 133 | 73 87 95 | 47 60 53 | $40 \\ 45 \\ 52$ | 38 42 45 |
| Sul | 16:0 18:1 18:2 | $100 \\ 100 \\ 100$ | 94 97 109 | $85 \\ 90 \\ 127$ | 71 85 99 | $55 \\ 68 \\ 71$ | $54 \\ 63 \\ 71$ | 49 56 62 |
| D₩ | 16:0 18:1 18:2 | $100 \\ 100 \\ 100$ | $89 \\ 96 \\ 112$ | $85 \\ 88 \\ 122$ | $73 \\ 87 \\ 100$ | $51 \\ 61 \\ 64$ | 42 51 55 | 4 4 5 |
| Control | $16:0 \\ 18:1 \\ 18:2$ | $100 \\ 100 $ | 96 95 112 | 88 86 122 | 55 65 74 | 32 37 39 | $22 \\ 24 \\ 27$ | $10 \\ 10 \\ 10 \\ 10 \\ 10 \\ 10 \\ 10 \\ 10 \\$ |

presence in 100 g of seeds as reported in earlier works (9,21).

The data on neutral oil are considered as pure triglyceride fraction because mono- and diglyceride fractions are negligible in quantity. Insignificant amount of mono- and diglyceride fractions suggests that the triglyceride molecule may remain absorbed on the enzyme and glycerol gets desorbed only after all the acyl groups are liberated. This mechanism is exactly opposite to one proposed by Hilditch (22) in the case of esterification of glycerol in ripening coconuts.

From Table II it is marked that among the three major fatty acids 16:0, 18:1 and 18:2, there is a gradual decrease of 18:1 in the control as well as in the treated seeds up to the third day of germination. On the other hand the amount of 18:2 acid is found to increase up to the third day of germination. After the third day the amount of 18:2 also begins to decrease. Experiments show that the major acids undergo a tremendous decrease after the sixth day of germination, confirming once again their intense metabolism in the control and in the treated seeds. Table I shows that the amount of neutral oil remains practically unconsumed during the first 24 hr of germination. In spite of the nonutilization of neutral oil during the early stage of germination, there is a variation in the composition of component fatty acids, suggesting a dynamic state of triglyceride. Simultaneous decrease or increase of 18:1 and 18:2 acyl group is assumed to be due to interconversion among the triglycerides of these groups in the germinating groundnut seedlings.

In the present investigation, the conversion of oleic acid to linoleic acid is assumed to occur during the initial stage of germination of the control and the treated seeds. The decrease in the amount of 18:1 is from 22.2 to 21.0 g, whereas the corresponding increase of 18:2 is from 17.0 to 19.0 g in 100 g control seeds on the first day of germination. These observations are in agreement with those of Vereshchagin and Ganieva (23) who reported that the conversion of oleic acid to linoleic acid occurred at the beginning of germination of cotton seeds between the first and the third day.

After the third day of germination it is observed that the neutral oil begins to metabolize. As shown in Table I, the 18:2 acid in control seeds decreases from 20.8 to 12.6 g between the third and the sixth day of germination. Hence it is assumed that the

TABLE IV Percentage of Oil in Cottonseed C-1622^a

| | | | irradi: 1ation i | | | | | radiate ation i | | 8 |
|------------------|---|------|---------------------|------|-----|---|------|--------------------|-----|-----|
| | 1 | 3 | 5 | 7 | 10 | 1 | 3 | 5 | 7 | 10 |
| % Oil Content | | 28.2 | 18.4 | 11.8 | 2.9 | | 30.8 | 18.4 | 7.4 | 1.6 |

Data from reference 23

process of interconversion of fatty acids is immediately operative under circumstances favorable to germination, and the metabolism of the neutral oil may be initiated with 18:2 acid. On the basis of this assumption, conversion of saturated acids to unsaturated ones may be occurring before their utilization during germination.

The conversion of palmitic acid to stearic acid is reported by Stumpf and Barber (24) in mitochondrial preparations of Avocado mesocarps. Radioisotope exchange studies by James (25) on castor leaves pointed out that interconversions of saturated and unsaturated acids occur on incubation of the leaves. The conversion of saturated to unsaturated acids may occur according to the mechanism suggested by Bloch (26), viz., Stearyl-CoA + TPNH + H^+ + $O_2 =$ Oleyl – CoA + H₂O + TPN⁺ . . . , and oleic acid is converted to linoleic acid subsequently as suggested before.

From the study of component fatty acids it may be concluded that first of all, the fatty acids are converted to 18:2 then metabolized during germination of control seeds. On account of the interconversion of the fatty acids, the amount of 16:0 in the present work decreases to 88 p.c.; that of 18:1 falls to 86 p.c.; 18:2 increases to 122 p.c. on the third day of germination of the control seeds (Table III). In case of seeds treated with growth regulators and with DW, the same trend is observed during early stages of germination. Thus, the present study gives a more conclusive evidence for the interconversion of the fatty acids than shown from results of Vereshchagin and Ganieva (23) during germination of cotton seeds. The data on the percentage oil and composition of fatty acid in triglyceride are given in Tables IV and V. The data in Table V are obtained from a graphic presentation of the results. It is from these results that the component fatty acids in 100 g cotton seeds

TABLE V Percentage Composition of Fatty Acids in Triglyceride of Cottonseeds Oil^a

| Acids | | Non germin | irradis ation i | | | | | rradiat nation : | | |
|--------------|----------------|----------------|--------------------|--------------|----------------|----------------|----------------|---------------------|----------------|----------------|
| | 1 | 3 | Б | 7 | 10 | 1 | 3 | 5 | 7 | 10 |
| 16:0 18:0 | 26.6 | $19.4 \\ 2.0$ | $18.7 \\ 2.3$ | 18.0 3.0 | 16.0 4.7 | $25.0 \\ 2.0$ | $25.7 \\ 2.6$ | $16.0 \\ 2.6$ | $14.0 \\ 4.7$ | 24.7 6.0 |
| 18:1 18:2 | $15.9 \\ 55.8$ | $15.0 \\ 59.6$ | $22.0 \\ 52.0$ | 20.0 49.0 | $25.0 \\ 40.3$ | $16.3 \\ 56.4$ | $18.0 \\ 52.7$ | $19.0 \\ 39.1$ | $29.3 \\ 37.0$ | $23.7 \\ 42.3$ |
| 18:3 | 0.0 | 4.0 | 5.0 | 10.0 | 14.0 | 0.3 | 1.0 | 23.3 | 15.3 | 3.8 |

* Data from reference 23.

TABLE VI Composition of Fatty Acids in 100 g Cottonseeds^a

| Acids | | Noni germina | rradia ation ir | | | | Ir germin | radiate ation i | | |
|-------|------|-----------------|--------------------|-----|-----|------|--------------|--------------------|-----|--------------|
| | 1 | 3 | 5 | 7 | 10 | 1 | 3 | 5 | 7 | 10 |
| 16:0 | 7.5 | 5.6 | 3.4 | 2.1 | 0.5 | 7.7 | 7.9 | 2.9 | 1.0 | 0.4 |
| 18:0 | 0.5 | 0.6 | 0.4 | 0.4 | 0.1 | 0.6 | 0.8 | 0.5 | 0.4 | 0.1 |
| 18:1 | 4.5 | 4.2 | 4.0 | 2.4 | 0.7 | 5.0 | 5.5 | 3.5 | 2.2 | 0.4 |
| 18:2 | 15.7 | 16.8 | 9.7 | 5.7 | 1.2 | 17.4 | 16.2 | 7.2 | 2.7 | 0.7 |
| 18:3 | 0.0 | 1.1 | 0,9 | 1.2 | 0.4 | 0.1 | 0.3 | 4.3 | 1,1 | \mathbf{T} |

Data from reference 23.

^b Traces

| Acids | g | Noni: ermina | | in da | - | ge | Irra rmina | adiate tion i | | 78 |
|-------|-----|-----------------|----|-------|----|-----|---------------|------------------|----|-----|
| | 1 | 3 | 5 | 7 | 10 | 1 | 3 | 5 | 7 | 10 |
| 16:0 | 100 | 75 | 45 | 28 | 7 | 100 | 103 | 62 | 13 | 5 |
| 18:0 | 100 | 120 | 80 | 80 | 20 | 100 | 133 | 83 | 67 | 17 |
| 18:1 | 100 | 93 | 95 | 57 | 17 | 100 | 110 | 70 | 44 | 8 |
| 18:2 | 100 | 107 | 61 | 36 | 8 | 100 | 93 | 41 | 16 | - 4 |

^a Data from reference 23.

have been calculated (Table VI), assuming no loss in weight of the seedlings during germination. A decrease in the amount of 18:1 from 4.5 to 4.2 g, i.e., from 100 to 93% between the first and the third day as shown in Table VII cannot account for the corresponding increase in 18:2 and the formation of 1.1 g of 18:3 not present on the first day of germination. During the same period 16:0 decreases considerably due to its conversion to 18:0. The increase in the amount of 18:0 is from 0.5 to 0.6 g only, whereas that of 18:2 is from 15.7 to 16.8 g, i.e., 100 to 107%. Considering the increase in 18:2 and 18:3, it seems reasonable that the decrease in both the 16:0 and 18:1 may be responsible for the said increase through their successive stages of interconversion. If one considers that up to the third day of germination, there may not be any change in percentage of neutral oil, the increase or decrease of any component fatty acids can be accounted for by their interconversion. On the fifth day of germination, the amount of 18:2 reduces to 61% of its original amount (Table VII). This indicates that metabolism of the neutral oil of cotton seeds occurs through 18:2 acid rather than through other fatty acids. The generalized view of interconversion of fatty acids was also perceived when the results of Huber and Zalik (27) and Zimmerman and Klosterman (28) on flax seeds during germination were expressed in 100 g flax seeds (9).

Figures 1 to 3 show data on change in the rate of interconversion and utilization of component fatty

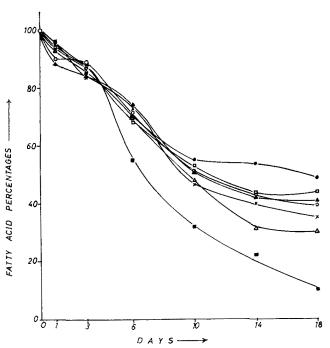


FIG. 1. Variation in the per cent of palmitic acid (16:0) with the period of germination. Treatments: \bigcirc GA, \triangle AA, \square SUC, \times MH, \bullet SUL, \blacktriangle DW, \blacksquare CONTROL. (Table IV.)

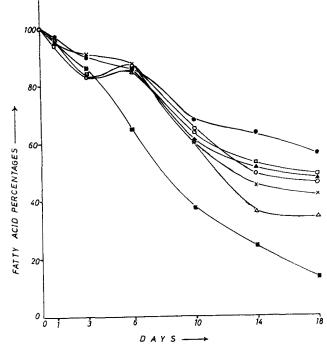


FIG. 2. Variation in the per cent of oleic acid (18:1) with the period of germination. Treatments: \bigcirc GA, \triangle AA, \square SUC, \times MH, \bullet SUL, \blacktriangle DW, \blacksquare CONTROL. (Table IV.)

acids in neutral oil with each treatment considered as 100 g in ungerminated seeds. These figures indicate that the rate of interconversion of fatty acids is affected to a certain degree by the action of growth regulators. It is minimum in Suc treated seeds, while in MH treated seeds it is higher, in comparison to

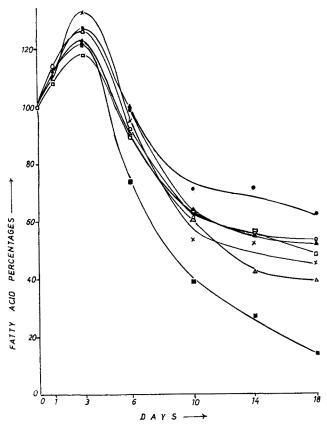


FIG. 3. Variation in the per cent of linoleic acid (18:2) with the period of germination. Treatments: \bigcirc GA, \triangle AA, \square SUC, \times MH, \blacksquare SUL, \blacktriangle DW, \blacksquare CONTROL. (Table IV.)

other treatments, up to the third day of germination. This is because these regulators may have varied effect on the enzymes responsible for the interconversion of fatty acids. As germination progresses the rate of utilization of the component fatty acids is also affected. This can be seen from the data given in Table III where the amounts of the major acids 16:0, 18:1 and 18:2 are shown to be reduced to 10, 13 and 14% of the original amounts, respectively, at the end of 18 days. Figures 1 to 3 show that the rate of utilization of the component acids undergoes reduction in comparison to the control seeds; the utilization rate is almost the same among treatments up to the 10th day of germination but differs afterwards. The rate of utilization of these major acids varies accord-

ing to the trend given below. Major acids utilization in AA treatment > MH >Suc or GA or DW > Sul. At the conclusive period of germination, the amounts of the major acids 16:0, 18:1 and 18:2 acid are found to be nearly 30, 34 and 39%, respectively, of their initial amounts in the AA treated seeds, while they drop to 39, 46 and 53 p.c. in the GA treated seeds. In Sul treated seeds, they are reduced to 49, 56 and 62% of the original amounts, respectively. Variation in the rate of utilization of component fatty acids in the treated seeds may be correlated not only with the growth but also with the other metabolic factors including the process of photosynthesis, as explained earlier. The results of the present investigation thus confirm the view that pregermination growth regulator treatments do affect the process of interconversion as well as the rate of utilization of component acids.

It can be concluded that the mitochondrial processes (29) involving acetyl-CoA units as well as nucleotides (TPNH and DPNH) necessary in the interconversion of fatty acids may be actively operating during the process of intense metabolism. These processes are slowed down in the treated seeds, indicating some sort of damage to mitochondria and cells of the seedlings.

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[Received January 3, 1968]