MATERIALS AND METHODS

EXPERIMENTAL PROCEDURE

A study of the pattern of enzymic activities and ascorbic acid metabolism in *Sesamum* seedlings was undertaken during the early stages of germination. The work was extended to the adult phase in *Sesamum* plants with the objective of having data on the enzymic activities as well as energy relations of these plants up to maturity. In order to study these the following experiments were carried out:

**Experiment I** : "The biochemical changes associated with the germination of *Sesamum indicum* cv. Kundla with special reference to sulfhydryl content and enzymic activities".

**Experiment II** : "Influence of exogenous application of ascorbic acid on enzymic activities and sulfhydryl content in *Sesamum indicum* cv. Limbdi during early stages of seed germination".

**Experiment III** : "The effect of photoperiod on growth, flowering and seed development as well as enzymic activities and sulfhydryl content of *Sesamum indicum* cvs. Limbdi and Kundla".
EXPERIMENT I

"THE BIOCHEMICAL CHANGES ASSOCIATED WITH THE GERMINATION OF SESAMUM INDICUM CV. KUNDLA WITH SPECIAL REFERENCE TO SULPHHYDRYL CONTENT AND ENZYMIC ACTIVITIES"

Seeds of sesame (Sesamum indicum L) cv. Kundla were incubated in sterilized petridishes (14 cm. diameter) lined with sterilized filter paper (Whatman No.1) in glass distilled water (DW). Germination was carried out upto 96 hours upto fully expanded cotyledonary leaves (Plate - 1,1*) in normal light and room temperature (30 to 32°C). The media was changed twice a day and the whole seedlings were taken for the following estimations at 24 hourly intervals upto 96 hours after germination. Methods used for biochemical estimations are given at the end of this chapter. The readings used to plot the graphs are means of three separate determinations and they are presented in Plates - 1 to 3.

(1) Percent moisture content
(2) Dry weight g./seed
(3) SH content as mg./g.fr.wt.
(4) Peroxidase activity as O.D./min./g.fr.wt.
(5) AA-FR-Peroxidase activity as O.D./20 min./g.fr.wt.
(6) Catalase activity as ml. O₂ evolved/min./g.fr.wt.
(7) Lipase activity as ml. of 0.1N sodium hydroxide used/g.cake/24 hours.
(8) Amylase activity as mg.starch digested/hr./g.fr.wt.
(9) Invertase activity as mg.glucose produced/30 min./g.fr.wt.
(10) Total sugar content as mg./g.fr.wt.
(11) Ascorbic acid (AA), Ascorbigen (ASG), Net ascorbic acid bound (NAB) as mg./g.fr.wt. and ascorbic acid utilization (AAU) as per cent AA utilized within 3 hours.

*The first figure indicates Plate No. and the following figure indicates the number on the plate. This method of presentation of data has been used throughout in this thesis.
EXPERIMENT II

"INFLUENCE OF EXOGENOUS APPLICATION OF ASCORBIC ACID ON ENZYMIC ACTIVITIES AND SULPHHYDRYL CONTENT IN SESAMUM INDICUM CV. LIMBDI DURING EARLY STAGES OF SEED GERMINATION"

Seeds of Sesamum indicum cv. Limbdi were incubated in sterilized petri-dishes (14 cm. diameter) lined with sterilized filter paper (Whatman No.1) in 40 ml. of (i) glass distilled water (DW) and (ii) equal volume of ascorbic acid solution (50 ppm). Germination was carried out for 96 hours upto fully expanded cotyledonary leaves in normal light and room temperature (30-32°C). The substrates were changed twice a day. The whole seedlings were taken for the following estimations at 24 hourly intervals upto 96 hours after incubation. Methods used for biochemical estimations are given at the end of this chapter. The readings used to plot the graphs are means of three separate determinations. Results are presented in plates - 4 to 6.

1. Percent moisture content
2. Dry weight g./seed
3. SH content as mg./g.fr.wt.
4. Peroxidase activity as O.D./min./g.fr.wt.
5. AA-FR-Peroxidase activity as O.D./20 min./g.fr.wt.
6. Catalase activity as ml. O₂ evolved/min./g.fr.wt.
7. Lipase activity as ml. of 0.1N sodium hydroxide used/g.cake/24 hours.
8. Amylase activity as mg. starch digested/hr./g.fr.wt.
9. Invertase activity as mg. glucose produced/30 min./g.fr.wt.
10. Total sugar content as mg./g.fr.wt.
11. Ascorbic acid (AA), Ascorbigen (ASG), Net ascorbic acid bound (NAB) as mg./g.fr.wt. and ascorbic acid utilization (AAU) as per cent AA utilized within 3 hours.
Methods for biochemical estimation are given at the end of this chapter.

EXPERIMENT III

"THE EFFECT OF PHOTOPERIOD ON GROWTH, FLOWERING AND SEED DEVELOPMENT AS WELL AS ENZYMIC ACTIVITIES AND SULFHYDRYL CONTENT OF SESAMUM INDICUM CV. LIMBDI AND KUNDLA"

A. Growth and Developmental Studies:

Seeds of Sesamum indicum cv. Limbdi and Kundla were sown in earthen-ware pots (9" in diameter). Each pot contained a loamy soil and farm-yard manure in 3:1 proportion. Pots were watered daily. In each pot 10 seedlings were sown in the beginning but later they were thinned down to 7 plants per pot by removing plants for fresh and dry weights. Manuring was done once a week at the rate of 1 gram per pot and the manure consisted of a mixture of ammonium sulfate (NH₄)₂SO₄ and superphosphate (2 to 1). When the seedlings had become well-rooted they were transferred to the three photoperiods namely ND, SD and LD as described in "Experimental methods".

Growth characters namely height, leaf and branch number of 15 plants selected by the method of random numbers were recorded at weekly intervals. Average values of 15 determinations are presented for the above-said characters in Plates 7 and 8.

Fresh weight and dry weight of root, stem, leaves, flower-buds and fruits were also recorded at weekly intervals.
They were taken in triplicate and mean value of 3 plants are presented here for dry weight.

Relative growth rate (RGR) was worked out as described under "Experimental methods" for each organ separately. Net assimilation rate (NAR) and leaf weight ratio (LWR) were also worked out and are presented. Rate of flowering was determined by counting the number of plants flowering every day. Number of fruits per plant were also recorded daily. 15 plants were selected for harvest data from each photoperiodic treatment by the method of random numbers. The following observations were recorded -- dry weight of root, stem, fruit and whole plant, number of fruits, total grain weight, total grain number and 1000-kernel weight. The data for the above mentioned characters are presented in Plates - 7 to 12.

B. **Metabolic and Enzymic Studies:**

Sampling was done for the following estimations at the various stages of growth and reproductive differentiation as well as grain maturation (Plate - 13) under three photoperiods as described under Experiment IIIA. Similarly samples from the subtending leaf at different developmental stages were also subjected to the following estimations --

1. SH content as mg./g.fr.wt.
2. Peroxidase activity as O.D./min./g.fr.wt.
3. AA-FR-Peroxidase activity as O.D./20 min./g.fr.wt.
Various stages of vegetative and reproductive differentiation as well as seed maturation of *Rosenbus Andicuim L.*
(4) Catalase activity as ml. $O_2$ evolved/min./g.fr.wt.
(5) Amylase activity as mg. starch digested/hr./g. fr.wt.
(6) Invertase activity as mg. glucose produced/30 min./g.fr.wt.
(7) Total sugar content as mg./g.fr.wt.
(8) Ascorbic acid (AA), ascorbigen (ASG), net ascorbic acid bound (NAB) as mg./g.fr.wt. and ascorbic acid utilization (AAU) as percent AA utilized within 3 hours.

Plants under LD condition remained vegetative so the terminal vegetative shoot apices and corresponding leaves were estimated along with the various stages of SD and ND plants. Methods of biochemical determinations are given at the end of this chapter, and the results are presented in this thesis in Plates - 14 to 46.

**EXPERIMENTAL METHODS**

**Fresh weight Dry weight and % moisture determination:** Seeds were weighed immediately after dissection and transferred to an oven at 80°C for 48 hours when a constant dry weight was recorded. The difference between fresh weight and dry weight was taken as moisture content and % moisture content was calculated on fresh weight basis. The dry weight, mean of 3 replicates, is expressed in all the cases in terms of g. per seed.

**SH:** The SH content is measured using colorimetric method of Grunert and Phillips (1949, 1951).
Reagents:
1. Sodium nitroprusside 2% solution
2. 0.067 M NaCN in 1.5 M Na₂CO₃ (15.9 g in 100 ml.)
3. Saturated sodium chloride solution - prepared at room temperature.

Procedure:
6 ml of NaCl is taken and to it is added 2 ml of the solution to be tested, 1 ml sodium nitroprusside solution and 1 ml Na₂CO₃–NaCN mixture. The optical density is measured at 520 μm (green filter) in a photoelectric colorimeter. Blanks are prepared in the same manner except that 2 ml distilled water is used instead of 2 ml test solution (blank 1) and 1 ml distilled water is used instead of 1 ml sodium nitroprusside.

The SH content per g.fr.wt. is calculated using the following regression prepared from standard cysteine:

\[ y = 0.0415 \times + 0.0019 \]

where \( y \) = concentration in mg.
\( x \) = O.D.

Peroxidase Activity: Peroxidase activity is determined by the method of Maehly (1954) and George (1953).

The sample is weighed, homogenized, made to a volume and centrifuged. The supernatant serves as a source of the enzyme. A reaction mixture is prepared by adding 2 ml phosphate buffer (pH 7.0), 1 ml of 20 mM aqueous guaiacol (0.22 ml guaiacol in 100 ml distilled water) and to this is
added one ml enzyme extract. A blank is run by preparing the reaction mixture in the above manner except that the enzyme is excluded and replaced with one ml distilled water. The scale of Klett Summerson Colorimeter is zeroed with blank mixture and the O.D. of the reaction is read. Then the scale of colorimeter is taken 25 divisions ahead from the point of O.D. noted for reaction mixture using blue filter (470 nm). At this stage the colorimeter switch is put off and 0.02 ml of 10 mM H₂O₂. (0.4 ml of 20 vol. H₂O₂ + 9.6 ml of distilled water) is added by dipping a thin rod in the cuvette containing reaction mixture and stop watch is started simultaneously. The time taken in seconds to reach the adjusted O.D. is noted. The results are calculated and enzyme activity is expressed in terms of O.D. of colour developed/min./g. fr. wt.

AA-FR-Peroxidase: Special peroxidase (AA free radical peroxidase) is assayed by the method of Gurevich (1963). The sample is weighed, crushed in ice cold distilled water and centrifuged. The supernatant is used for enzyme assay. To 2 ml of the supernatant 1 ml of ascorbic acid (5 mg/ml) and 1 ml of saturated solution of o-dinitrobenzene* and 0.1 ml of 20 vol H₂O₂ are added. Blank contains all the above solutions except 0.1 ml H₂O₂, which is replaced.

*1 g. o-dinitrobenzene was suspended in approx. 100 ml of glass distilled water, kept in boiling water bath for 15 minutes and filtered in cold. The volume is made upto 100 ml. This colourless solution is stored at 30°C.
with glass distilled water. The solutions are allowed to stand at room temperature 26 ± 1°C for 20 minutes. The optical density of the yellow colour (formed due to the reduction of o-dinitrobenzene to o-nitrophenyl-hydroxylamine by the free radical of ascorbic acid) developed was recorded using blue filter on Klett Summerson colorimeter against the blank. The activity was calculated and expressed as O.D. of the colour developed per 20 ml per g. fresh weight of the material.

**Catalase Activity:** Catalase activity is assayed by the manometric technique of Chance and Maehly (1955). The material is weighed and crushed in a mortar with pinch of sterilized sand and calcium carbonate. The extract is made to 20 ml and transferred to a reagent bottle. 5 ml of H₂O₂ (10 volume) and 2 ml phosphate buffer (pH 7.0) are taken in a polythene tube (1" x 1") and carefully transferred to the reaction bottle. The bottle is connected to the manometer at zero. The mixture is shaken for 2 minutes and the difference in reading which gives the volume of oxygen liberated is noted. The results are calculated per minute of time and per gram fresh weight of the tissue, which expresses the rate of catalase activity.

**Lipase Activity:** Lipase activity is determined by the method of Urs at al., (1962). The sample is repeatedly (2-3 times) crushed and extracted with acetone for the preparation of the cake. The powder is defatted, residue is dried in an incubator maintained at 36 ± 1°C for 24 hours.
100 mg of this cake is weighed and suspended in 10 ml citrate phosphate buffer (pH 8.0). To it, is added 1 ml of triacetin (as substrate) and 1 ml of toluene (to prevent infection). The reacting system is incubated at 36 ± 1°C for 24 hours. The enzyme activity is stopped by adding 25 ml absolute alcohol. The amount of acid produced is titrated with 0.1 N NaOH to a distinct pink colour using one ml of 1% solution of phenolphthalein as the indicator. A blank was run. It had all the contents except the enzyme-cake. The activity is expressed as ml of 0.1 N sodium hydroxide used per g. cake/24 hours.

Amylase Activity: The material is weighed, homogenized, made to a volume and centrifuged. The supernatant served as the enzyme source. The reaction is carried out for 30 minutes at 30°C using 1-2 ml of 1-5% starch substrate as the case may be. The control consists of enzyme inactivated by boiling, in boiling water bath (100°C) for 45 minutes.

In control as well as reaction sets 0.5 ml of 10% acetic acid and 0.5 ml of 0.1 N KI and a drop of 3 vol H₂O₂ is added and it is allowed to develop colour for 30 minutes at 30°C. The excess of iodine is removed by giving repeated changes of chloroform. The intensity of the colour is compared using control set on left side and reaction set on right side of a Kruss colorimeter.

The amount of residual starch is determined by the method of Chinoy (1939) and thus amylase activity is
calculated as starch hydrolyzed per hour per g. fr. wt. of tissue.

**Invertase Activity:** The method of Hatch and Glasziou (1963) is used for invertase assay. A weighed amount of material is crushed in a mortar using cold distilled water with sterilized sand and made up to a volume of 10 ml. Out of this, only 1 ml aliquot is added to a reaction mixture containing 1 ml of 0.25% sucrose and 1 ml citrate buffer (pH 5.5). The reaction is carried out at 30°C for 30 minutes. The enzyme activity is inactivated by adding 2 ml of 5% perchloric acid. The solution is made up to a constant volume (10 ml). 1 ml of this final solution is taken to develop colour by anthrone method (please see McCready et al., 1950 - Total Sugars). The colour is read on a photoelectric colorimeter. The enzyme activity is calculated using regression formula and it is expressed as mg. glucose produced/30 minutes/g.fr.wt.

**Sugars:** The method of estimation of sugars is that of McCready et al., (1950). Plant tissue is dropped in boiling 80% alcohol and crushed thoroughly and left overnight to extract sugars. After centrifuging the supernatant is hydrolysed with 1 N HCl in a boiling water bath. The solution is neutralised, cooled, made up to a volume and analysed for the total reducing sugars using anthrone.

0.2 g. of anthrone is dissolved in 100 ml of 95% sulfuric acid (A.R.) and cooled in refrigerator. Solution to be analysed and anthrone are mixed in 1:2 proportion and
heated over a water bath at 100°C for 7.5 minutes. The furfuryl groups of sugars combine with anthrone to form bluish green or green complex. The optical density of this coloured complex is read in the colorimeter, using a red (660 mµ) filter, the reading is compared with a standard regression curve of known concentrations of sugar (glucose) and the concentration of total sugar in the unknown solution is calculated. Regression formula calculated for photoelectric colorimeter:

\[ y = 0.8102x - 0.00035 \]

where \( y \) is sugar concentration in mg.

and \( x \) is optical density.

Ascorbic Acid (AA), ascorbigen (ASG), ascorbic acid utilization (AAU) and Net ascorbic acid bound (NAB) from a given plant sample, were determined by the following modified method of Chinoy et al., (1969):

I. Determinations using a photoelectric colorimeter:

Reagents:

1. Metaphosphoric acid - (i) 3% solution.

   Metaphosphoric acid - (ii) 15% solution.

These solutions can be kept in the refrigerator for 4 days.
2. **Buffer solution - A**: 10.55 g. of Citric acid are dissolved in 100 ml. of 1 N NaOH.

**Buffer solution - B**: 31.65 g. of Citric acid are dissolved in 100 ml of 3 N NaOH.

3. **Buffered HPO<sub>3</sub>**: Two volumes of 3% HPO<sub>3</sub> are mixed with one volume of buffer solution - A (pH - 3.6). **Buffered HPO<sub>3</sub> is freshly prepared** every day.

4. **Ascorbic acid solution for AAU**: 10 mg. of ascorbic acid are dissolved in glass distilled water, which was boiled, cooled and saturated with CO<sub>2</sub>(D.W.). The volume of the solution was made up to 100 ml. and stored in an amber coloured bottle at 3-5°C. Ascorbic acid solution is always freshly prepared.

5. **Standardised dye solution**: 10 mg. of 2,6-dichlorophenolindophenol (BDH) are dissolved in deionized water at 80°C, cooled, its volume made up to 200 ml. and its strength is standardized by the addition of either deionized water or more concentrated dye solution to it until 5.0 ml of the dye solution when mixed with 2.0 ml of deionized water gives a reading of 390 on the scale of the photo-electric colorimeter. This standardization of the dye solution is done every time just before starting estimations.

**Standard Curve**: Ascorbic acid solutions of concentrations ranging from 0.01 to 0.09 mg./ml. are prepared from a stock solution by dilutions with cold CO<sub>2</sub>-saturated glass distilled water to the requisite concentration.
1 ml. aliquot of each AA solution is mixed with 1.0 ml. of buffered HPO$_3^-$ solution and 5.0 ml. of Standardised Dye Solution and the reading on the scale of photo-electric colorimeter is recorded using a green filter. 10-20 colorimetric readings are taken for each AA solution and these are converted into optical density (O.D.) by multiplying with 0.002 (which is the calibration factor of Klett Summerson Colorimeter).

Regression of AA concentration on O.D. is calculated and the position of the trend line determined from the following regression equation:

$$y = 0.1103 - 0.14x$$

where $y$ = concentration of AA in mg.

$x$ = optical density.

**EXTRACTION:** Weighed sample of plant material is placed in a mortar and covered with 1 - 2 ml. of cold CO$_2$-saturated glass - distilled water (D.W.) and a pinch of purified silica sand. It is then quickly homogenized and the contents are transferred to a Pyrex test-tube. The mortar and pestle are rinsed two to three times with 1 - 2 ml. of cold CO$_2$ - saturated glass - distilled water, transferred to the test-tube and the volume of the homogenate is made upto 15 ml. with D.W. (CO$_2$ saturated).

The homogenate is divided into three parts:

(i) 3 ml. for the estimation of AA;
(ii) 3 ml. for the estimation of ASG; and
(iii) 3 ml. for the estimation of AAU and NAB
Determination of ascorbic acid: 3.0 ml. cooled buffered HPO₃ is added to 3.0 ml. of the original homogenate and after thorough shaking a 2.0 ml. aliquot of the mixture is diluted with 5.0 ml. of D.W. and the pointer on the colorimetric scale is adjusted at '0' for the turbidity factor. 5.0 ml. of standard dye solution are added to another 2.0 ml. aliquot of the same mixture and the colorimetric reading is noted.

Calculation: Colorimetric reading is converted into optical density (O.D.) by multiplying with 0.002 (the calibration factor of Klett Summerson Colorimeter). Using the regression formula $y = 0.1103 - 0.14x$, the concentration of AA in 1.0 ml. of the original extract is calculated. As each 2 ml. aliquot contains 1.0 ml. of the original homogenate the concentration of AA/g. fr. wt. of the material can be calculated as follows:

$$A = \frac{a \cdot v}{w} \cdot 1000$$

where $A =$ AA content of the sample in mg./g. fr. wt.
$a =$ AA in mg./ml. of the original homogenate
$v =$ Total volume of the original homogenate
and $w =$ weight of the plant sample taken for analysis (in mg.).

DETERMINATION OF ASCORBIGEN (ASG): 1.5 ml. of 15% HPO₃ (Reagent No.1(i)) is added to 3.0 ml. of the original homogenate and the mixture kept in a water bath at 75°C for 15 minutes for hydrolyzing ascorbigen. After cooling, the system is buffered to pH 3.6 by adding 1.5 ml. of the
Buffer Solution - B thus increasing the volume of the mixture to 6.0 ml. To a 2.0 ml. aliquot of this buffered hydrolyzed extract 5.0 ml. of D.W. are added and the pointer on the colorimetric scale is adjusted at 'O' for the turbidity factor. 5.0 ml. of the Standard Dye Solution are added to another 2.0 ml. aliquot of the buffered - hydrolyzed extract and the colorimetric reading is noted.

Concentration of AA in 2.0 ml. of the buffered-hydrolyzed extract is calculated from the regression equation and the value of free AA in 1.0 ml. of the homogenate, which was determined previously, is subtracted from it to obtain the AA equivalent of ascorbigen in 1.0 ml. of the homogenate. Ascorbigen content per g. fr. wt. is then calculated as follows:

\[ B = \frac{v(b - a)}{w} \times 1000 \]

where \( B \) = AA equivalent of ascorbigen in mg. per g. fr. wt. of the sample.

\( b \) = AA (mg.) in 2.0 ml. of buffered - hydrolyzed extract

\( a, v \) and \( w \) are the same as in the case of free AA

**ASCORBIC ACID UTILIZATION (AAU) AND NET ASCORBIC ACID BOUND (NAB)**

3.0 ml. of ascorbic acid solution for AAU (10 mg./100 ml.) are added to 3.0 ml. of the original homogenate and the mixture is incubated at 30°C ± 2°C with thorough shaking.
after every 10 minutes. Two aliquots of 3.0 ml. each are taken out separately for the above two determinations after 3 hours.

**Determination of AAU:** 2.0 ml. of buffered HPO$_3$ are added to 2.0 ml. of the incubated solution immediately after incubation and after thorough shaking a 2.0 ml. aliquot is mixed with 5.0 ml. of D.W. and the pointer on the colorimetric scale is adjusted at '0' for the turbidity factor. Another 2.0 ml. aliquot is mixed with 5.0 ml. of the Standard Dye and the colorimetric reading is noted, which is then converted into O.D. value by multiplying with 0.002 and the AA content of 2.0 ml. aliquot of the buffered incubated solution (i.e. 0.5 ml. of the original homogenate) is calculated from the regression equation. The value of AA in mg. obtained from the regression equation is multiplied by 2* to obtain the value of AA in mg. per 1.0 ml. of the original homogenate left unutilized immediately after incubation period of 3 hours. The calculation of AAU as per cent of the total AA originally present in the incubated solution is as follows:

*Multiplication by the factor - 2 is necessary because 2.0 ml. aliquot of the mixture after the addition of equal volume of buffered HPO$_3$ to the incubated solution contains only 0.5 ml. of the original homogenate. This value of unutilized AA is deducted from the total AA content (in mg./ml.) of the original homogenate before incubation (which includes the endogenous AA in mg./ml. as well as 0.1 mg./ml. of AA added exogenously) to determine the amount of AA utilized in 1.0 ml. of the original homogenate in 3 hours.
\[ C_1 = \frac{v(a + 0.1) - 2c}{w} \cdot 100 \]

where \( C_1 \) = Ascorbic acid (in \%) utilized per g.fr.wt. during a given period of incubation. 

\( c \) = Amount of AA in mg. left over in 2.0 ml. of the buffered - incubated solution (i.e. 0.5 ml. of the original homogenate).

**Determination of Net Ascorbic Acid Bound (NAB):** 2.0 ml. of 15% \( \text{HPO}_3 \) are added to 4.0 ml. aliquot of the incubated solution and the mixture is placed in a water bath at 75°C for 15 minutes for hydrolysis of the complex. After cooling, the mixture is buffered at 3.6 by adding 2.0 ml. of Buffer Solution - B bringing the total volume to 8.0 ml. 5.0 ml. of D.W. are then added to a 2.0 ml. aliquot of this buffered - hydrolyzed extract and the pointer on the colorimetric scale is adjusted at '0' for the turbidity factor. Another 2.0 ml. aliquot of the above solution is pipetted out and after the addition of 5.0 ml. of the Standard Dye Solution colorimetric reading is noted; which is converted into O.D. value and the AA content in the aliquot is calculated from the regression equation.

The value of AA (in mg.) obtained from the regression equation is multiplied by 2, because 2.0 ml. aliquot taken for colorimetric reading contains only 0.5 ml. of the original homogenate. Substracting the value of AA of 1.0 ml. of the original homogenate utilized before hydrolysis from the above value gives the amount of AA released by hydrolysis of the AA-MM complex (in mg.ml.).
The amount of AA complexing with macromolecules per g. fr. wt. can be calculated for the aliquot taken immediately after incubation period of 3 hours as follows:

$$D_1 = \frac{2v(d-c)}{w} \times 1000$$

where $D_1$ = mg. of AA released from the complex per g. fr. wt. of the material

$d$ = mg. of AA in 2.0 ml. of the incubated hydrolyzed buffered solution (i.e. in 0.5 ml. of the original homogenate).

Photoperiodic treatment: Seeds of Sesamum were sown in earthenware pots (9" in diameter). Photoperiodic treatment was started when the seedlings had become well rooted. Then the total pots were divided in three equal parts and they were transferred to the following 3 photoperiods - (1) natural condition called as normal day (ND) having a mean photoperiod of about 12.5 hours; (2) short day (SD) treatment, receiving 8 hours of natural light. After completion of which the whole lot is transferred to the dark chamber; (3) Long day (LD) treatment. In LD treatment continuous light is given where natural day-light is supplemented by flouroscent tubelights.

Growth analysis: Methods of growth analysis developed by Gregory (1921, 1926) are used to determine the growth behaviour of plants. Weekly growth measurements of height, number of branches and leaves on the main stem are taken for 10 - 15 plants per treatment as the case may be.
Whole plant sample method of Gregory is utilised for this purpose. Three replicates of plants are selected by the method of random numbers at weekly or fortnightly intervals as the case may be. These plants are uprooted carefully to minimize damage to the roots and wrapped in wet cloth before bringing to the laboratory. The plants are thoroughly washed by water to remove soil and then gently pressed between blotting sheets to remove moisture on their surface. Plant parts viz. root, stem, leaf and bud are separated and fresh weight of each part is recorded. These parts are then placed in paper bags, and transferred to an electrically operated oven at 60°C. for a period of one week for complete and uniform drying. The dry weights are then recorded.

Relative growth rate: Relative growth rate is determined as difference between the Naperian logarithms of dry weights of successive weekly samples as shown by Blackman (1919). The formula of relative growth rate is as follows:

\[
R.G.R. = \frac{\log_e W_1 - \log_e W_0}{t}
\]

Net assimilation rate: From the data of dry matter production of the whole plant and leaf, net assimilation rates are calculated using Gregory's formula (1926):

\[
\frac{W_1 - W_0}{\log_e L_1 - \log_e L_0} = \frac{L_1 - L_0}{\log_e L_1 - \log_e L_0}
\]

where \(W_0\) and \(W_1\) represent the successive dry weights of the whole plants; \(L_0\) and \(L_1\) represent the successive dry
weights of the leaves of the corresponding samples. Net assimilation rates are determined on the basis of unit dry weight of leaf instead of unit leaf area.

Leaf weight ratio: The following formula is used for determining the leaf-weight ratio:

\[ \frac{L_1 - L_0}{\log_e L_1 - \log_e L_0} \times \frac{W_1 - W_0}{\log_e W_1 - \log_e W_0} \]

where \( L_0 \) and \( L_1 \) represent the successive dry weights of the leaves and \( W_0 \) and \( W_1 \) represent the successive dry weights of the whole plants.

Relative rate of Biosynthesis or enzymic activity: Considering that the enzymic reactions and other metabolic processes are exponential in nature Blackman's formula can be applied to obtain the rates of enzymic reactions or biosynthesis of a metabolite by taking the difference between the Naperian logarithms of the enzymic activities or metabolic levels at two given stages of development such as vegetative, reproductive stages as follows:

\[ r = \frac{\log_e m_r - \log_e m_v}{t} \]

where \( r \) = relative rate of biosynthesis of a metabolite or enzymic activity.

\( m_r \) = metabolic level or enzymic activity at a given stage of reproductive differentiation.

\( m_v \) = metabolic level or enzymatic activity at a given stage of vegetative differentiation.

\( t \) = time interval (Days).