VARIATION IN THE RATE OF RESPIRATION OF A GERMINATING SEED

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Extensive work on respiration has been done by several investigators, and many types of respirometers have been designed and recommended for estimating this activity in plants. The kinds of micro-respirometers constructed [Osterhout and Haas (1917) and Lund (1919)] and the methods recommended appear to be too complicated. The indicator method of Osterhout (1918) although efficient needs very careful handling. Davis (1925) has designed an apparatus which works on the manometer principle but the estimation of carbon dioxide is done only after 24–48 hours of respiration. The several germinating seeds likely to be introduced at a time, the possibility of accumulation of carbon dioxide in the vicinity of seeds and the long period after which the estimation is made happen to be the drawbacks in this case. A more recent contribution to this subject is by Brown (1942) who has designed an apparatus and studied the rate of gaseous exchange in the seed and cotyledons of *Cucurbita pepo*. The smallest quantity recorded by him happens to be 1/100 c.c. The duration of each one of his experiments was 48 hours during which period four estimations are made, these being at intervals of 18, 24, 42 and 48 hours from the time of starting the experiment. According to the author each estimation takes about 10 minutes, or more for greater accuracy, and necessitates certain corrections in the volume for the time lost during estimation. In all these investigations the readings are taken at long intervals and no effort seems to have been made as yet to record this activity at very short intervals. It was this which made the author to study this activity in the germinating seeds. For this work a special kind of respirometer had to be designed on the 'float and manometer' principle (Krishna Iyengar, 1942 *b*), and this in combination with the optical lever constructed by the author makes it possible to record very small volume of gases. The simple construction, high magnification, easy handling, efficient working and lastly direct observation were the points in view during the construction of the apparatus (Fig. 1). Strong caustic soda solution is used for the ready absorption of carbon dioxide evolved. Miller (1931) is of opinion that 'the use of a strong solution of an alkali in the apparatus has a disadvantage, since the ready absorption of carbon dioxide by the alkali introduces large changes in pressure within the closed apparatus thereby affecting respiration'. In the present case the bulk of respiring

material is very small, the period of investigation is short, the difference in pressure is insignificant and even then provision is made to bring up the pressure to normal. The following is an account of the apparatus and the observed variation in the rate of respiration.

Apparatus and Its Working

The trough of water (A) is meant to maintain a constant temperature in the specimen tube by directly absorbing any heat evolved during respiration. A film of oil is introduced to remove

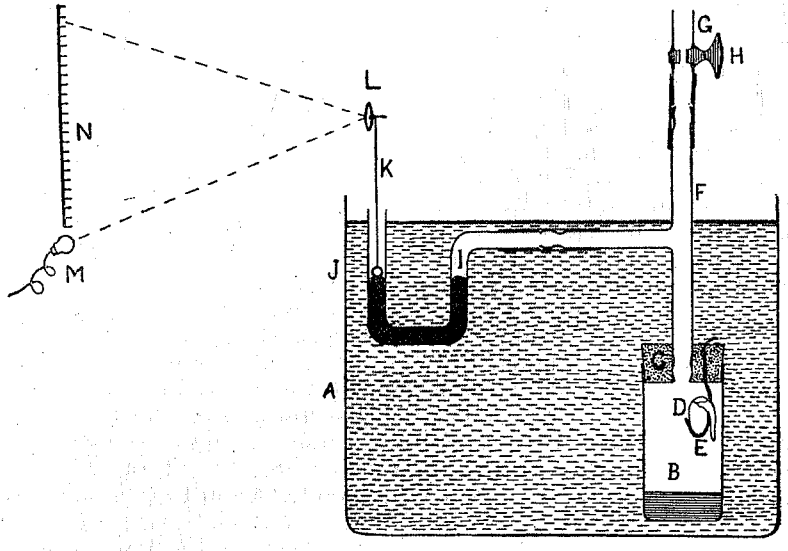

Fig. 1. Diagram of the micro-respirometer. *A*, Trough of water with a film of oil ; *B*, Specimen tube with caustic soda solution ; *C*, Rubber stopper ; *D*, Germinating seed ; *E*, Clamp ; *F*, 'T' tube; *G*, One-way glass tube ; *H*, Stop-cock ; *I*, 'U' tube with mercury ; *J*, Float ; *K*, Silk fibre ; *L*, Optical lever ; *M*, Light ; *N*, Scale.

the possibility of any fall in temperature due to evaporation of water. The tube (B) contains strong caustic soda solution at its bottom to absorb readily any carbon dioxide liberated during respiration. The germinating seed (D) is wrapped in moist blotting paper and fixed in the clamp (E). The wire from the clamp projects into the water outside. This is a device to remove immediately any heat evolved during respiration. The stopcock (H) allows or cuts off communication with the exterior, the latter being necessary before taking readings. During respiration the seed makes use of the oxygen in the chamber and liberates carbon dioxide. The ready absorption of the latter by caustic soda solution results in the rise of mercury in the closed end of the U-tube

(I). The float is attached to the short arm of the optical lever (L), the other arm being the beam of light. A galvanometric mirror mounted on the balance wheel of a watch and capable of revolving on the fine bearings forms the most important part of this optical lever. A stand with rack and pinion arrangement facilitates the proper adjustment of the optical lever and of the beam of light on the scale before recording is started. Any small depression of the float will result in the movement of the mirror in the clockwise direction, with the consequent upward movement of the focussed beam of light. Shifting the place of attachment of the float towards or away from the mirror results in a higher or lower magnification respectively. The author has made use of a mirror of 2 metre focal length, and the minimum distance at which the beam of light was focussed happens to be 2 metres. When very high magnification is necessary the distance between the scale and the mirror can be increased (this depending on the length of the room and the power of illumination) or the distance between the place of float attachment and the fulcrum reduced. By increasing the distance between the scale and the mirror to 3 metres and by reducing the distance between the fulcrum and the place of attachment to 2 mm. it is possible to have a magnification of 3,000. The movement of the beam of light projected on the scale (N) indicates the change in volume due to respiration of the seed. The shortest distance that could be observed without the help of a lens happens to be 1/40 inch. Since the diameter of the U-tube employed happens to be 4 mm. the movement of the beam of light through this distance will indicate a change in volume by nearly 1/384000 c.c., when the magnification happens to be 3,000. But for the present investigation the magnification employed was 400, thus making the smallest volume about 1/51200 c.c. for 1/40″ distance on the scale. While drawing the graphs the rates obtained from the movement of the beam at minute intervals have been doubled or quadrupled to enable a proper reproduction of the figures after reduction.

Materials and Method

The room temperature was constant during the brief period of observation in each case. The apparatus was tested before it was set up for observation. Control experiments were set up to detect the variation of pressure, if any, due to moisture. For this purpose a piece of wet blotting paper, instead of a germinating seed, was introduced into the clamp, and recording was started after an interval of an hour or more. The readings taken at minute intervals and with a very high magnification indicate that the pressure inside remains constant during a sufficiently long period. Care was taken to enable the chamber to attain saturation in humidity by introducing extra quantity of wet blotting paper for wrapping the seed. It may be stated that since the tube is a closed chamber there is every possibility of the air in the chamber reaching a stage of saturation in humidity since the quantity of

moisture lost from the blotting paper and from the seedling due to transpiration and respiration will be very much greater than the quantity of water absorbed by the surface of alkali solution during the same period. Depending on the quantity of water available in the blotting paper the stage of equilibrium in the saturation can be maintained for several hours at a stretch. Fresh solution of alkali was used for each experiment for the absorption of carbon dioxide as quickly as possible. Since the distance between the solution and the seed is very short the possibilities are in favour of small volumes of carbon dioxide settling on the solution with minimum delay, only to be readily absorbed by it.

Germinating seeds of *Dolichos lablab*, *Cicer arietinum*, *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* were selected for studying the rate of respiration. The seed coat was carefully removed in all, except *Zea Mays*, and the germinating seed or young seedling was carefully washed and weighed before this was used for the experiments. The germinating seed was left in the apparatus for a period of about $\frac{1}{2}$ hour or more before recording was started. Necessary magnification was adjusted and the readings were taken at intervals of a minute for a period of $\frac{1}{2}$ to 1 hour or more. The particulars connected with the author's observations on the respiration of a germinating seed of each plant are presented in a tabular form, and the variation in the rate of respiration has been represented in the form of graphs given below.

Observations

The following tabular statements give an idea of the weight of the germinating seed, duration of experiment, magnification employed, volume of oxygen utilised, room temperature and other particulars.

The five graphs introduced in this paper show the rates of respiration in the germinating seeds of different plants. The figures 2, 3, 4, 5 and 6 are the graphs of the germinating seeds of *Dolichos lablab*, *Cicer* sp., *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* respectively. From these figures and the data connected with each it is noticed that respiration does not go on at a uniform rate but is given to fluctuations in its rate from time to time. There are periods of high activity alternating with those of reduced ones, these resulting in the major fluctuations in the graphs. Respiration proceeds on at a high rate only for a few minutes, the period of high activity being generally between 6 to 10 minutes or more depending on the kind of young seedling. During the periods of major fluctuations there are minor fluctuations in the rate, these occurring at intervals of 1 to 2 minutes, or more. There is appreciable difference between the highest and lowest rates of this activity, the former being at times four to ten times the latter as is seen in the graphs.

Data connected with the respiration of a germinating seed of Dolichos lablab (*Fig.* 2)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·80	0·80	0·70	0·65	0·50	0·65	0·70	0·55	0·65	0·60	6·60	76° F.
2nd ,, ..	0·60	0·70	0·65	0·90	0·90	0·75	0·90	0·95	..	0·75	7·20	,,
3rd ,, ..	1·05	1·00	0·75	0·85	0·60	0·85	0·75	0·80	1·00	1·10	8·75	,,
4th ,, ..	0·60	0·70	0·60	0·50	..	..	..	..	..	..	2·40	,,

Movement at the end of 33 minutes of activity .. 24·95

Weight of the germinating seed .. .. 0·812 gm.
Period of activity .. .. .. 33 minutes
Distance travelled by the beam .. .. 24·95 inches
Magnification employed .. .. ×400
Fall in the mercury column .. .. 0·156 cm.
Volume of oxygen absorbed .. .. 0·0195 c.c.

Data connected with the respiration of a germinating seed of Cicer arietinum (*Fig.* 3)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·425	0·925	0·85	0·95	0·80	0·75	0·55	0·35	0·25	0·15	6·00	74·5° F.
2nd ,, ..	0·150	0·150	0·25	0·30	0·75	0·20	0·40	0·275	0·925	0·85	4·25	,,
3rd ,, ..	0·300	0·600	0·50	0·60	0·60	0·75	0·85	0·300	1·250	..	5·75	,,
4th ,, ..	..	0·400	0·10	0·20	0·30	0·25	0·15	0·200	0·200	0·15	1·95	,,
5th ,, ..	0·150	0·050	0·15	0·15	0·10	0·05	0·10	0·100	0·150	0·10	1·10	,,
6th ,, ..	0·100	0·150	0·05	0·15	0·15	0·15	0·15	0·200	0·250	0·25	1·60	,,
7th ,, ..	0·250	0·550	0·50	0·85	1·10	0·90	0·95	1·000	0·800	0·80	7·70	,,
8th ,, ..	0·900	0·900	0·85	0·90	..	..	..	..	..	..	3·55	,,

Movement at the end of 72 minutes of activity .. 31·90

Weight of the germinating seed .. .. 0·258 gm.
Period of activity .. .. .. 72 minutes
Distance travelled by the beam .. .. 31·9 inches
Magnification employed .. .. ×400
Fall in the column of mercury .. .. 0·199 cm.
Volume of oxygen absorbed .. .. 0·025 c.c.

Data connected with the respiration of a germinating seed of Phaseolus vulgaris (*Fig.* 4)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·55	0·55	0·575	0·575	0·65	0·75	0·75	1·15	0·90	0·525	6·975	75° F.
2nd ,, ..	0·675	0·55	0·575	0·625	0·65	0·375	0·775	0·60	0·55	..	5·375	,,
3rd ,, ..	0·70	0·80	0·85	1·00	0·95	1·075	1·075	0·55	0·70	0·45	8·150	,,
4th ,, ..	0·55	0·50	0·325	0·25	0·325	0·650	..	..	..	..	2·600	,,

Movement at the end of 35 minutes of activity .. 23·100

Weight of the germinating seed .. .. 1·045 gm.
Period of activity .. .. .. 35 minutes
Distance travelled by the beam .. .. 23·10 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. 0·144 cm.
Volume of oxygen absorbed .. .. 0·018 c.c.

Data connected with the respiration of a germinating seed of Pisum sativum (*Fig.* 5)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·15	0·375	0·45	0·15	0·475	0·35	0·35	0·05	0·15	0·55	3·05	74° F.
2nd ,, ..	0·35	0·525	0·225	0·25	0·275	0·425	0·60	0·45	0·35	0·225	3·675	,,
3rd ,, ..	0·425	0·45	0·40	0·70	0·55	0·45	0·50	0·35	0·45	0·30	4·575	,,
4th ,, ..	0·50	..	..	..	..	..	..	..	..	..	0·50	,,

Movement at the end of 31 minutes of activity .. 11·80

Weight of the germinating seed .. .. 0·455 gm.
Period of activity .. .. .. 31 minutes
Distance travelled by the beam .. .. 11·80 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. 0·074 cm.
Volume of oxygen absorbed .. .. 0·0093 c.c.

Data connected with the respiration of a germinating seed of Zea Mays (*Fig.* 6)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·625	0·575	0·75	0·575	0·70	0·575	0·75	0·875	0·875	0·825	7·125	74° F.
2nd ,, ..	0·875	0·85	0·85	1·00	..	..	0·575	0·70	0·625	0·725	6·20	,,
3rd ,, ..	0·60	0·95	0·675	0·725	0·575	0·95	0·575	0·50	0·50	0·60	6·65	,,
4th ,, ..	0·60	0·55	0·80	..	0·40	0·425	0·25	0·675	0·65	0·40	4·75	,,
5th ,, ..	0·55	..	..	..	..	..	..	..	..	..	0·55	,,
Movement at the end of 38 minutes of activity .. 25·275												

Weight of the germinating seed .. .. 0·275 gm.
Period of activity .. .. .. 38 minutes
Distance travelled by the beam .. .. 25·275 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. 0·158 cm.
Volume of oxygen absorbed .. .. 0·0198 c.c.

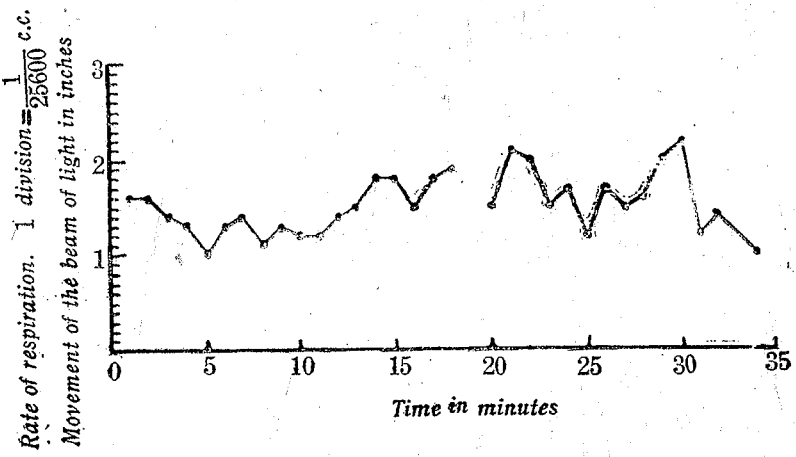

Fig. 2. Graph to show the variation in the rate of respiration in *Dolichos lablab*. ×2.

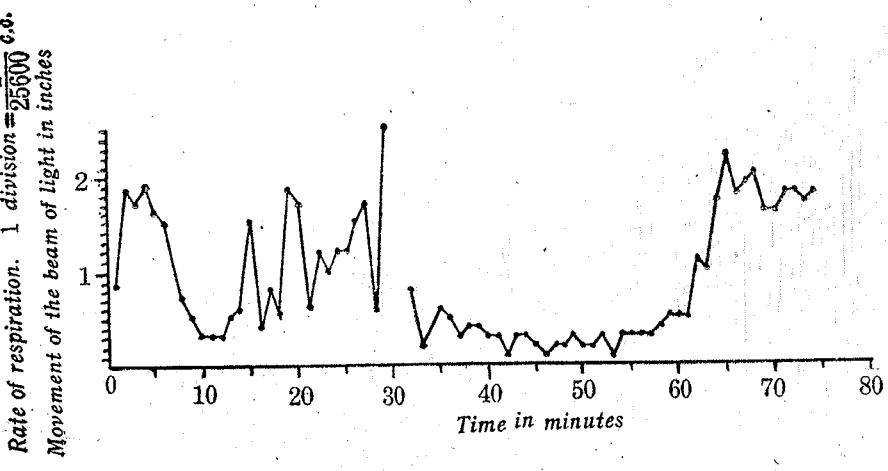

Fig. 3. Graph to show the variation in the rate of respiration in *Cicer*. ×2.

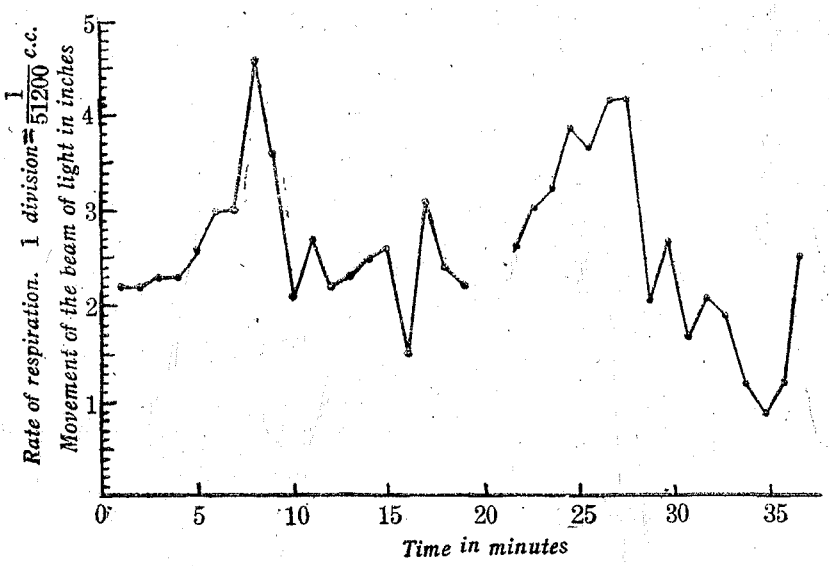

Fig. 4. Graph to show the variation in the rate of respiration in *Phaseolus vulgaris*. ×4.

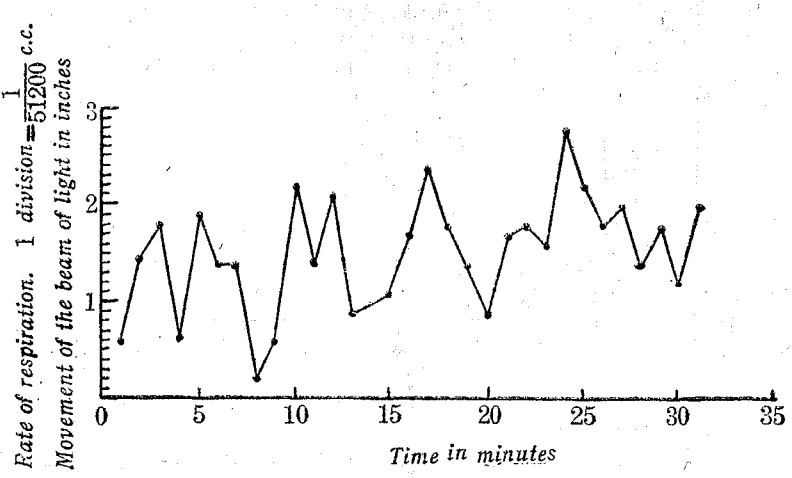

Fig. 5. Graph to show the variation in the rate of respiration in *Pisum sativum*. ×4.

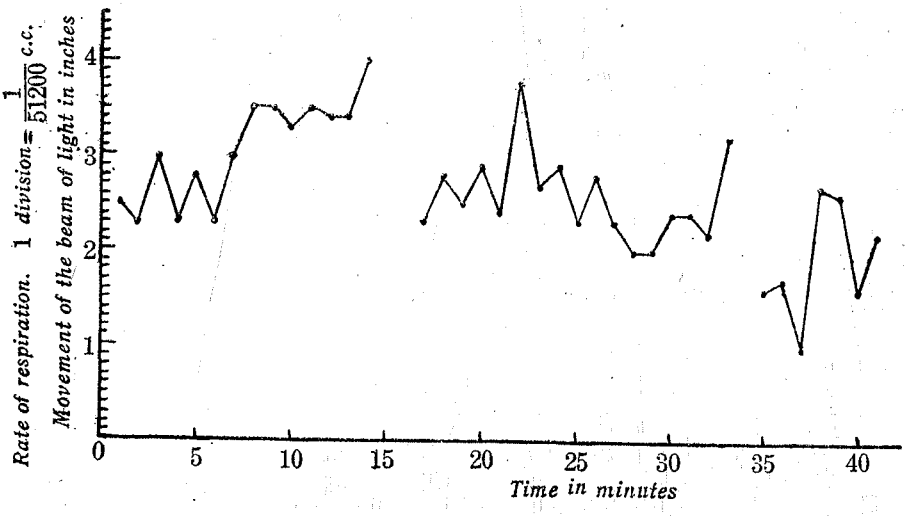

Fig. 6. Graph to show the variation in the rate of respiration in *Zea Mays*. ×4.

Discussion

From the above account it is found that notwithstanding constant external conditions there are momentary and periodical variations in the rate of respiration. A detailed enquiry into the external factors affecting respiration is out of place since the conditions are maintained almost constant during the brief periods of observation.

The experiments of Hopkins (1926) on potato illustrate not only the influence of temperature but also the effect of diastase and the accumulation of sugar on this activity. In the present case one is inclined to believe that a temporary or momentary and purely local variation in temperature within the tissues is a possibility and that this might have its own share in the variation of the activity of diastase and consequently momentary variation in the rate of respiration as shown by the minor fluctuations in the graph.

Role of moisture in respiration and its variation has been clearly explained by Bailey (1918) and other investigators. The author's observations on the leaf movements and water absorption (Krishna Iyengar, 1942 *b*) indicate that in many plants the water-content of the plant body will be varying even at short intervals of part of a minute. If similar fluctuations occur in the tissue of the germinating seed the possibilities are in favour of noticeable fluctuations in the rate of respiration also.

According to Miller (1931) the quantity of oxygen in the intercellular spaces of fruits and other plant parts primarily due

to poor gaseous exchange is often considerably below that of the air outside and may thus in some cases be limiting factor in the respiration of these parts. It is not improbable that momentary accumulation of carbon dioxide or the depletion of oxygen or both might temporarily affect respiration directly, and indirectly by affecting enzymatic activity resulting in the small oscillations at short intervals of a minute or less.

Finally the tone of the tissue may also count a great deal in deciding the rate of respiration; and its variation depends on several metabolic activities. The author's study of photosynthesis (Krishna Iyengar, 1942 *a*), leaf movement, water absorption and transpiration (Krishna Iyengar, 1942 *b*) and even growth (Krishna Iyengar, 1942 *c*) points towards the occurrence of variations in the rates of all these activities in several plants even when the external conditions are almost constant, indicating an oscillation in the rates of all these at short as well as at long intervals. Respiration shows similar fluctuations. In all these activities an active period will invariably be followed by a period of depression. These indicate the existence of possible fluctuation in the tone of the tissue from time to time, with its appreciable influence on the rates of all the vital activities. In conclusion it may be stated that while minor variations in the rate of respiration may be due to several factors like the temporary and purely local variation in the temperature due to respiration, fluctuations in the water-content, enzymatic activity and the available quantity of respirable material at a particular time and concentration of oxygen or carbon dioxide in the intercellular spaces, major variations which occur at intervals of 6 to 10 minutes or more can only be attributed to the possible fluctuations in the tone of the living tissue from time to time.

Summary

1. The germinating seeds of *Dolichos lablab*, *Cicer arietinum*, *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* were selected for studying respiration.

2. A special micro-respirometer was designed and the readings were taken at minute-intervals and the graphs were drawn.

3. A single germinating seed was taken at a time and the rate of respiration recorded.

4. The graphs represented indicate the occurrence of major and minor fluctuations in the rate of this activity, the former generally occurring at intervals of 6 to 10 minutes or more (depending on the nature of the seed, time of the day, kind of plant, internal activities, etc.), while the latter at intervals of a minute or two—at times even less than a minute.

5. Some of the factors like temporary or momentary and purely local variations in temperature due to respiration, fluctuating water-content, enzymatic activity, available quantity

of respirable material at a particular time and variation in the concentration of carbon dioxide and oxygen in the intercellular spaces of the tissue are probably responsible for the variation at short intervals.

6. The periodic variation in the tone of the living tissue is an important factor, and this seems to be reflected in the alternating periods of high activity and depression seen not only in respiration but also in several other vital activities of the plants.

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