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Physiological Studies during Vernalization in Rice*

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Introduction. The term vernalization is a comparatively new one and its original equivalent in Russian is *Jarovizotie.* The term signifies (a) a stage of development during the germination of seeds; (b) the process developed by Lyssenko by which the flowering phase of a plant is forced earlier than usual by certain pre-treatments of the seed.

Theory of Vernalization. The main theoretical conceptions on which vernalization is based are (1) growth and development are two independent processes; (2) morphological features are no indication of development; (3) plants have different stages of development.

Growth and development are two independent processes that take place in a plant. The length of the vegetative period is not fixed for a plant. It is determined by a set of external factors though an individual factor can hinder the growth of a plant. Factors determining development are different from those determining growth and they are not antagonistic to each other. The time required by a plant to flower is determined by certain factors whose nature and magnitude are different for different plants. These factors may be allowed to act on germinating seeds or on growing plants. The duration of such stimulants varies in like manner both for the germinating seed and for the green plant. The process of sexual development is determined even in the germinating seed.

Lyssenko mainly recognises five stages of development. The plants follow strict sequence and cannot proceed to the next stage unless the previous stage is completed. Of the five stages only two are completely understood. The first one is "vernalization stage" or "thermo-stage" and the second one is "photo-stage". The third stage, according to Kraevai and Kiricenko (1935) is associated with gametogenesis.

Photoperiodism. Klebs was the first to recognize the fact that the flowering duration in plants is not inherent in them but can be altered. Light is one of the factors. Garner and Allard (1920) divided plants into the following classes. (1) Long-day plants which progress quickly towards maturity under an artificially prolonged day. (2) Short-day plants which progress quickly towards maturity under shortened day. (3) Plants which are indifferent to light period.

On-set of flowering. In addition to the theory of photo-periodism there are two more theories regarding the on-set of flowering—(1) Hormone theory and (2) Carbon-nitrogen ratio theory. Lyssenko refutes the conception that there is antagonism between development and growth of plants. The main

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difference between Lyssenko and others lies in the material used for experiments. The former dealt with vernalized seeds and others dealt with green plants. The latter in their attempts to make the plant flower earlier shortened the assimilation period and thus brought about poorer vegetative growth. Lyssenko experimented with vernalized seeds and found that provided the required amount of darkness was given to the vernalized seed, the plant came to flower earlier even in continuous illumination. Lyssenko is of opinion that C: N ratio is only a result of the on-set of flowering and not the cause of it. Since the state of vernalization does not spread from part to part of a plant, the hormone theory does not hold good.

Vernalization in Rice. Ukrainskii (1934) found that the reduction of the length of day to 12 hours accelerated flowering by six days. Hence rice is concluded to be a short day plant. Ossewarde (1935) found that the plants of the two weeks treatment were 2–7 days earlier than the control. Haig (1934) in Ceylon found that the treatment for six to 10 days shortens the duration of rice when compared with dry sown seeds. The reduction in duration is mathematically significant but not economic.

Bio-Chemical studies. Biochemical processes relating to vernalization were studied only in Russia. Demkovsky (1932) found a general increase of enzyme activity and also a change in the inter-relation between different groups of enzymes. He expects to derive an indirect method to determine the stage of seed vernalization by changes in the enzyme complex. Rancan (1933) made a comparative study of the changes in the activities of diastase, protease, peroxidases and catalase, on winter wheat. Catalase showed a double maximum on the twentieth and twentyfifth days and the maxima were followed by a sharp fall. The study of Rubin and Naumova (1934) showed that there was a correlation between the energy of plant development and the action of enzymes, particularly that of catalase.

Material and method. The experiments were conducted on the strain of paddy G. E. B. 24 and four other strains obtained from Coimbatore Paddy Breeding Station. The seeds were sterilised in mercuric chloride solution and soaked in water for 18 to 20 hours. The water was then drained off and the moisture adhering to the seeds removed. They were then placed in dishes and kept in a cool chamber. The temparature of the chamber was maintained between 10° and 20°C. When the seeds appeared dried up, a small quantity of water was sprinkled over them. By careful adjustment of moisture the further growth of the seedling was prevented. One set of seeds was kept in a chamber which was illuminated continuously by a 500 Watt bulb. Another set was kept in a dark chamber. The temperatures of the two chambers did not differ very much. The seeds were subjected to this treatment for three weeks. The individual flowering duration of the plant from these seeds was determined by observation in the field and it was found that the flowering duration of the control was 101 days after transplanting while that of the seeds vernalized in darkness was 96·2 days. The difference though significant is not economic.

Experiments on Diastase and Catalase :— The changes in the quantity of diastase and catalase during the process of vernalization in G. E. B. 24 were followed. Diastase was estimated by pulverising the seeds and preparing 1% water extract. 10 cc. of 1% starch solution buffered at pH 4. 6 was added to 10 cc. of the extract and the enzyme action was allowed to proceed for 1 hour in a water bath maintained at 40°C. The reducing sugars formed were estimated by Shaffer and Hartman's micro method. The milligrammes of glucose so formed represent the diastase activity in 10 cc. of the extract or its equivalent of 0˙ 1 gm. of the seeds. From this the total activity in 100 seeds is calculated. The catalase activity was determined by pulverising 25 seeds with $CaCO_3$. This was placed in one arm of Heinicke's tube and in the other 5 cc. of neutralized Merck's H_2O_2 was placed. They were mixed and shaken at the rate of 25 shakings per minute. The quantity of oxygen evolved at the end of 5 minutes was taken as the measure of catalase after being reduced to N. T P. The total catalase activity for 100 seeds was then calculated.

Diastase :— The diastase present in the seeds during the vernalization process is greater than that in un-germinated seeds. In appearance the vernalized seeds show no difference from the ordinary seeds except for the small crack in the seed coat at the region of the embryo. The diastase present in the seeds that are vernalized in light is greater than the quantity present in the seeds vernalized in darkness. The data are presented below :-

TABLE I. Quantity of diastase in vernalized seeds:—
(Figs. in mgm. of glucose for 100 seeds).

Day of vernalization.	Vernalization in	
	light.	darkness.
3	58·08	67·24
4	183·04	44·00
5	159·44	25·68
6	92·56	23·60
7	114·40	60·88
11	148·20	30·28
24	132·48	—
25	152 76	30·28

The two sets of G. E. B. 24 seeds that were vernalized in light and darkness were then placed for germination in trays after 40 days of the treatment. All seeds germinated and they were analysed for diastase. The data are presented below :—

TABLE II. Quantity of diastase in vernalized seeds when they germinate.

Hours after soaking.	Seeds vernalized in		Control untreated seed.
	light	darkness	
48	197·75	93·85	112·60
72	435·20	408·15	317·52
96	608·70	666·40	636·48
120	913·10	998·60	558·04

The seeds treated in light show a larger diastase content up to the third day; later the seeds treated in darkness show a larger content. The quantity present in the treated seeds are considerably greater than those in the untreated seeds.

Catalase. The sampling of the seeds for determination of catalase was done on the same day as that for diastase determinations, so as to find out the course of change in the two enzymes in relation to each other. The data are presented in the following table:—

TABLE III. Quantity of catalase in vernalized seed:—

Days of vernalization.	Vernalization in	
	light.	darkness.
3	24·58	7·10
4	18·95	7·44
5	20·67	8·13
6	11·84	7·78
7	14·90	11·18
11	10·84	7·45
24	16·20	9·79
25	12·81	9·78
26	11·46	9·44
37	9·16	10·17

It is found that the quantity of catalase in seeds treated in light is very high on the third day, but it slowly falls until on the 37th day it is dwindled to 1/3 the original content. The catalase present in the seeds treated in darkness is almost steady. However, the quantity is less than that in light treatment in the initial stages and the two are almost equal after 37 days of treatment.

The quantities of catalase present in the treated seeds when they germinate were followed. The data are given below:—

TABLE IV. Quantity of catalase present in the vernalized seeds when they germinate.

Hours after soaking	Vernalized in		Control untreated seeds.
	light.	darkness.	
48	7·61	10 15	36·36
72	39·34	48·64	88·73
96	85·12	95·29	146·38
120	114 62	134·14	180·02

The catalase content of the seeds vernalized in light is less than that of the seeds treated in darkness. The comparison between the catalase content of the vernalized seeds and that of the control is interesting. The quantities of catalase present in the treated seeds are far less than those of the control.

Discussion. The experiments show that the seeds undergoing vernalization treatment contained larger amounts of diastase and catalase than

ungerminated seeds. Control seeds when they germinate on moist blotting-paper grow rapidly and in 48 hours the radicle grows to nearly 0·1 to 0·2 inches length. At this stage of growth the diastase content of 100 seeds is 112·60. The seeds undergoing vernalization treatment have shown more than this quantity of diastase though with regard to growth they have only burst open the seed coat near the embryo and are not growing any further. The diastase content of the seeds vernalized in light is very high and there are daily fluctuations. The diastase content of the seeds vernalized in darkness is far less than that of the light vernalized seeds. When the vernalized seeds were germinated on moist blotting paper after 40 days' treatment the higher diastase content of light vernalized seeds is maintained only up to the third day and later the darkness vernalized seeds take the lead. Both the treatments have effected a greater diastase content throughout the course of germination than that of untreated seeds.

Changes in the quantity of catalase in the vernalized seeds are similar to those of diastase. During vernalization in light the seeds show a very large increase in catalase in the initial stages but it slowly decreases. The seeds that are vernalized in darkness show almost a steady catalase content. When the seeds are kept for germination there is increase in catalase in both the lots. The seeds treated in darkness show slighily a larger amount of catalase than the ones treated in light. Both of them show considerably low contents of catalase as compared to the control. Therefore it may be concluded that while the vernalization treatment causes an increased diastase content in the germinating seeds it impairs the catalase production. The experiments show that the enzyme complex in the seeds undergoes a change during vernalization. The change is seen during the course of the treatment as well as when the seeds germinate after the treatment. A detailed study of the enzyme complex at different stages of vernalization may be useful to determine the degree of vernalization.

Summary. The vernalization treatment has reduced the flowering duration of rice by about 5 days.

Both diastase and catalase increase during the vernalization process. The treatment in light has caused greater increase than that in darkness. In the former treatment catalase decreases slowly until it is equal in both. When the seeds are germinated the increase in diastase is greater and that in catalase less than those of untreated germinating seeds.

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