The Journal of the Indian Botanical Society

Vol. XLIX 1970 Nos. 1-4

THE PHYSIOLOGY OF AGEING IN RICE SEEDS*

By S. M. Sircar

Bose Institute, Calcutta-9

(Received for publication on April 19, 1970)

Dormancy and viability are two important phenomena that influence the life span of a seed. The principal features responsible for longevity of seeds are presumably developed while the embryo matures and subsequently undergoes a period of rest or dormancy. Considerable

Dr. S. M. Sircar

attention has been paid to the factors that initiate the process of germination and hormonal regulation of the process has been visualized.

* Presidential address, Indian Botanical Society, January 1970.

The life span of seeds are variable and may extend from less than a year to more than 200 to 300 years. The mechanism relating to the ageing of seeds is not precisely known although it has been possible to a limited extent to extend the life span by controlling the seed moisture and storage temperature and by the application of chemical regulators. Some of the effects of ageing in seeds are accumulation of toxic substances, degradation of enzymes, nuclear damage and chromosome breakage. The question arises what mechanisms are responsible for these effects of ageing in seed and whether these could be controlled.

Ageing is a problem of considerable significance in rice seeds, it commences after the formation of the embryo. According to the nature of a variety there is a brief span of inactivity of the embryo when it remains in dormant condition but there are varieties also showing vivipary. One of the problems facing the rice breaders in India is the difficulty of maintaining valuable breeding stocks of viable seeds. Investigation on the possibility of prolonging the life span of the seed in storage is an important consideration in agricultural practice.

Seed germination of the winter variety of rice shows the presence of primary dormancy for about 6 weeks after harvest in December followed by normal germination of 100 per cent up to June–July, thereafter as the seeds age gradual loss of viability and fall in respiration, enzyme activity and vigour of seedling growth occur till they become completely non-viable in course of a year. Mitra (1969) reported decline in germination from 58 per cent in October to complete failure in December and progressive fall in the seedling vigour during the same period (Table I). Some of the seeds failing to germinate in October were stimulated to germinate by treatment with GA for 48 hours. With further ageing GA, however, failed to induce germination in such dormant seeds. This would mean that inhibitory factors are likely to be produced in these seeds in large excess which could not be overcome by GA. The respiration rates of dormant and sprouted embryos and endosperms tend to fall during the transition from viability to non-viability in December (Table I). The rates are, however, higher after germination than those of the dormant ones indicating that the germination inhibitors in dormant seeds also inhibit respiration. This is further evident from the fact that the respiration rates of the GA stimulated sprouted embryo and endosperm are similar to those of normally germinated ones. α-amylase activity in both germinated and dormant embryos and endosperms depleted gradually with age but after GA treatment the activity is raised in both up to the end of October only. On the other hand phosphatase activity in the endosperm remained more or less constant till November, then it declined but in the embryo there was progressive reduction during this period. The dormant embryo showed very little change in the activity during the successive months but GA treatment increases the activity of both embryo and endosperm up to a limited period, then it declines with age. It thus appears that GA acts as a germination stimulator which can counteract the effect of the inhibitor at the critical stage of losing

TABLE I

Physiological changes of rice seeds cv. Bhasamanik during ageing and loss of viability (after Mitra, 1969)

	September	October		November		December	
		1	15	1	15	1	15
Per cent of germination after 72 hours (normal)	67·0	58·0	51·2	33·2	15·3	4·6	Nil
GA (10^{-2} ppm) treatment of dormant seeds for 48 hours	..	88·0	70·6	55·6	25·0	10·3	Nil
Seedling vigour after 72 hours germination:							
Root growth (cm.) ..	..	2·13	1·65	1·02	0·77	0·31	Nil
Shoot growth (cm.) ..	..	1·32	0·95	0·68	0·52	0·11	Nil
GA (10^{-2} ppm) treatment of dormant seeds for 48 hours:							
Root growth (cm.) ..	..	3·05	2·12	1·10	0·55	0·21	Nil
Shoot growth (cm.) ..	..	1·51	1·30	0·82	0·31	0·10	Nil
Respiration rates $\mu l/O_2/hr/mg$ dry wt. after 72 hours germination:							
Embryo ..	..	7·04	7·01	5·40	2·26	1·06	Nil
Endosperm ..	..	0·62	0·48	0·36	0·36	0·31	Nil
GA (10^{-2} ppm) treatment of dormant seeds for 48 hours							
Embryo ..	..	7·21	5·18	3·70	3·01	1·00	Nil
Endosperm ..	..	0·55	0·39	0·30	0·25	0·20	Nil
Dormant seeds:							
Embryo ..	..	4·09	2·01	1·39	0·84	0·21	0·19
Endosperm ..	..	0·21	0·11	0·11	0·13	0·10	0·07
α-amylase activities (mg maltose released in 3 mins.) after 72 hours germination:							
Embryo ..	..	0·540	0·480	0·458	0·400	0·312	Nil
Endosperm ..	..	1·350	1·312	1·304	0·560	0·370	Nil
Dormant seeds:							
Embryo ..	..	0·172	0·152	0·065	0·059	0·048	0·032
Endosperm ..	..	0·680	0·511	0·357	0·364	0·288	0·066
GA (10^{-2} ppm) treatment of dormant seeds for 48 hours:							
Embryo ..	..	0·702	0·513	0·412	0·418	0·356	Nil
Endosperm ..	..	1·180	1·031	0·651	0·612	0·488	Nil
Phosphatase activities mg phosphorus released/mg protein after 72 hours germination:							
Embryo ..	..	34·0	24·2	21·2	15·1	10·3	Nil
Endosperm ..	..	33·8	32·5	33·0	34·0	17·1	Nil
GA (10^{-2} ppm) treatment of dormant seeds for 48 hours:							
Embryo ..	..	48·0	35·1	28·3	21·2	11·0	Nil
Endosperm ..	..	38·1	37·5	36·5	36·5	16·0	Nil
Dormant seeds:							
Embryo ..	..	5·13	4·0	5·0	5·0	3·01	5·0
Endosperm ..	..	24·5	20·1	24·1	23·2	20·5	20·0

viability but at a later stage in December it fails to neutralize the inhibitor. Seeds stored for more than one year were found to have lost viability, but they were not dead as both embryo and endosperm of even 5 years old were respiring at a rate slighty lower than the normal one (Sircar and Biswas, 1960). The failure of rice seeds to germinate one year after harvest is due to the formation of inhibitors which are mostly located in the grain, husks contribute little to the non-viability of the seeds (Dey and Sircar, 1968 *a*). A warm humid tropical climate in India provides unique opportunity to seeds with initial moisture content for the formation of inhibitors which are in most cases the degradation products of storage material.

These inhibitors are known to cause metabolic blocks to enzyme functions which otherwise become activated immediately upon imbibition and the effects of the inhibitors are expressed during germination, respiration and growth of seedling. Retardation of germination rate is proportional to the progressive accumulation of inhibitors and their precursors that may arise after the commencement of seed ripening. In order to determine whether there is causal relationship between the presence of various constituents in seeds in storage and the stage of after-ripening, it is necessary to investigate how far the state of after-ripening period is correlated with the inhibitor level. The presence of phenolics—coumarin, ferulic and sinapic acids—were detected mostly in the seed (Dey *et al.*, 1968; Sircar, 1969; Sircar and Dey, 1967; Dey and Sircar, 1968 *a*, *b*). But the phenolics are not directly involved in the loss of viability as the concentration of phenolics remains fairly high both in the viable, dormant and non-viable seeds (Table II). The phenolic concentration attained a very high level when the seeds became completely non-viable (Bhattacharya, 1969). Supra-optimal level of indole compounds was found to act as one of the germination inhibitors (Dey and Sircar, 1968 *a*). In addition, Dey and Sircar (1968 *b*) reported the presence of abscisic acid (ABA) in the embryo and very little in the endosperm. These inhibitors were found to be present in large concentration in the seeds losing viability with age while viable seeds showed absence or presence in very low concentration. The ABA present in the non-viable rice seed was found to depress the α-amylase activity in the endosperm, stop seed germination and reduce the straight growth of wheat coleoptile sections. The inhibitor ABA was also found to show interaction with gibberellic acid (GA) in reversing the GA induced amylase activity and GA at higher concentration partially overcomes the suppression of the inhibitor (Dey and Sircar, 1968 *b*). It does not seem to have any significant effect on the protease but depresses the phosphatase activity interfering the release of phosphate for oxidative phosphorylation (Sircar, 1967).

Sarkar (1967) demonstrated that germination of rice seeds is phytochrome-controlled. This was also indicated from the effects of red and far-red light on seeds losing viability with age. Seeds of more than one year old lost viability completely but after treatment with red light germinating capacity of 20 per cent seeds

TABLE II

Changes in total phenolics (mg/100 gm dry powder against standard curve of tannic acid) of rice seed cv. Bhasamanik during ageing and loss of viability (after Bhattacharjee, 1969)

Date of sampling, 1969	Stages	Acid	Basic	Neutral
January 15	Primary dormancy	1·25	1·92	1·60
February 15	,,	2·03	2·41	2·53
March 15	Germinated	1·89	1·42	2·29
April 15	,,	1·52	1·31	1·80
May 15	,,	1·42	2·52	1·86
June 15	,,	1·49	1·42	1·70
July 15	,,	2·01	1·42	1·90
August 15	,,	2·24	2·09	2·45
September 15	Leading to non-viability	2·59	2·13	2·94
October 15	,,	3·07	3·59	3·14
November 15	,,	3·92	4·01	3·42
December 15	,,	4·56	4·92	4·02

revived. The red and far-red light control was studied in relation to the synthesis of inhibitor level. Preliminary results showed that both age and exposure to far red increased the IAA oxidase activity in the endosperm while in the embryo the activity was less in both red and far-red light. The reduction of IAA oxidase activity in the embryo suggests the presence of the oxidase inhibitor. Both red and far-red light-treated seeds also showed the presence of inhibitors. The nature of the inhibitors and the enzyme systems in different wavelengths in relation to ageing is now being studied.

These results have led us to elaborate studies on the loss of viability of rice seeds with ageing on two aspects. Firstly, the mode and the function of the inhibitor complex and the factors associated with their formation. What biochemical reactions and the precursors are involved in the process of synthesis of the inhibitor complex? This problem is based on the fact that the embryo after formation does not germinate immediately and undergoes a period of rest for after-ripening known as primary dormancy. The important consideration will be to determine whether any of the inhibitors is synthesised during this period and to pinpoint biochemical reactions that lead to the synthesis of the inhibitor. Subsequently what happens to inhibitors when the

embryo after dormancy assumes growth? When the inhibitor complex starts again accumulating in the embryo with ageing? Secondly the investigations have been initiated to study the mechanism of the action of the inhibitor complex on the process of germination.

Since germination begins immediately after imbibition of water and resumption of enzyme action, the pertinent question would be to determine the enzyme action through nucleic acid metabolism. Accordingly the action of ABA, the inhibitor identified in the rice seed on the nucleic acid metabolism of viable and non-viable seeds has been studied by Dr. B. Dey in collaboration of Dr B. B. Biswas of Radio Chemical and Plant Biochemistry Laboratory of the Institute. The results (unpublished) so far obtained indicate that C^{14} leucine was not incorporated into the non-viable embryo indicating the failure of protein synthesis, but in the endosperm an appreciable amount of incorporation was detected. In order to find out the state of nucleic acid synthesis C^{14} uridine and C^{14} thymidine were used and the results show that these were not incorporated in the embryo of non-viable seeds but in the endosperm incorporation was obtained. Pretreatment of viable seeds with ABA greatly inhibited the incorporation of C^{14} uridine into RNA, the range of inhibition depends upon the concentration of ABA. But ABA did not prevent the incorporation of C^{14} uridine in the endosperm.

In order to overcome the failure of nucleic acid synthesis in the non-viable embryo, germination stimulators were used. Amongst them kinetin treatment resulted incorporation of C^{14} leucine and C^{14} aspartic acid in non-viable seeds but there was no morphological response of germination. C^{14} uridine and C^{14} thymidine were also incorporated in non-viable embryo in presence of kinetin suggesting the embryonic axis of non-viable seeds is capable of synthesising nucleic acid and protein in presence of kinetin. It was also found that in non-viable embryo C^{14} thymidine incorporation into DNA did not increase with increasing time period in contrast with the viable ones. When kinetin was added to non-viable embryo, incorporation of C^{14} thymidine into DNA increased 2·5 fold and the linearity with time was more or less maintained as in the case of viable embryo. These results are suggestive that cytokinins have a role in the induction of DNA synthesis in non-viable embryo but there is no revival of germination possibly there may be some defect or arrest in DNA synthesis and DNA may not be appearing in strands in the non-viable seeds. This abnormality in DNA strand would obviously reflect in the chromosome breakage which has been reported in the past by several workers (Abdalla and Roberts, 1968) in the cells during ageing of seeds. They have suggested that during ageing seeds accumulate chromosome damage when the frequency of such aberrant cells exceeds some critical level, the embryo is no longer capable of germination. It would then seem that nuclear damage is involved in normal ageing, then question arises what mechanism is involved in the production of the damage. In comparing the X-ray and mutagen-induced nuclear damages in old

seeds, several investigators have suggested the accumulation of auto-mutagenic substances in the seeds which are responsible for inducing chromosomal breakage and nuclear damage. But Abdalla and Roberts (1968) were unable to demonstrate mutagens in the species investigated. This would raise question whether the endogenous inhibitor ABA induces chromosomal aberration for the loss of viability of rice seeds. Further work on this line is now in progress.

REFERENCES

ABDALLA, F. H., AND E. H. ROBERTS. 1968. Effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans and peas during storage. *Ann. Bot.* **32**: 119–136.

BHATTACHARYA, J. 1969. Phenolic constituents of rice seeds during development, and germination and storage. *Rep. U.S.P.L.* 480 *Project* (unpublished).

DEY, B., AND S. M. SIRCAR. 1968 *a*. Viability and germination of rice seeds. Role of endogenous auxin levels. *Indian J. agric. Sci.* **38**: 477–485.

————. 1968 *b*. The presence of an abscisic acid-like factor in non-viable rice seeds. *Physiologia Pl.* **21**: 1054–1059

——, P. K. SIRCAR, AND S. M. SIRCAR. 1968. Phenolics in relation to non-viability of rice seeds. *Intern. Symp., Dept. of Botany, Calcutta University, Calcutta* 57–64.

MITRA, S. 1969. *Changes in the Chemical Constituents of Rice and Wheat Seeds During Germination and Their Significance in the Growth of the Embryo.* D.Phil. Thesis, Calcutta University, Calcutta.

SARKAR, K. K. 1967. *Physiological Studies of Wild Rice.* D.Phil. Thesis, Calcutta, University, Calcutta.

SIRCAR, P. K. 1969. *Isolation of Naturally Occurring Growth Substances from Tropical Plants.* D.Phil. Thesis. Part III. Calcutta University, Calcutta.

SIRCAR, S. M. 1967. Biochemical changes of rice seed germination and its control mechanism. *Trans. Bose Res. Inst.* **30**: 189–198.

————, AND M. BISWAS. 1960. Viability and germination inhibitor of the seeds of rice. *Nature, Lond.* **187** : 620–621.

——, AND B. DEY. 1967. Dormancy and viability of the seed of rice. *Proc. Symp. Phys. Ecol. Biochem. of Germination, Greifswald* D5. 969–973.