SUMMARY & CONCLUSIONS
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The efforts are being constantly made in our country to step up the production of food grains, especially the cereals which are responsible for satisfying the hunger of ever-increasing population. Apart from this population problem, we have several others: weather conditions unfavourable for the long-term storage and not so infrequent droughts appearing every or every other year. These facts when put together led us to concentrate on two points:

1. the improvement of seed storage, and
2. making use of seeds which are rendered useless due to partial loss of germinability and vigour in the productivity program.

In the first, seed being a living output its storage in one or other form is unavoidable, since preservation of seeds, physical and physiological qualities, is a prime aim of good storage, its relevance to the success of a seed programme cannot be overemphasised. The highest quality level for each species is the theoretical maximum attained under that complex maze of conditions evoking the most favourable interactions between the genetic makeup of the seed and the environment under which it is produced, harvested, processed and stored.

Cereal seeds which are commonly called the better storers do deteriorate on exposure to the typical Indian storage condition. These seeds (un)fortunately being produced in mass have received a little attention to store them in a greater quantity. Hence, studies were undertaken, especially on cereals, to find out the proper storage method.
and environment, and to understand, to localise, to prevent or to reduce the fundamental process of deterioration and to make use of deteriorated seed lots.

Keeping these views in mind, work was carried out on three major cereal crops, viz, wheat (Triticum aestivum L. cv C-306), maize or corn (Zea mays L. cv African Tall), and bajra or pearl-millet (Pennisetum typhoides L. cv GHB-27).

The experiments were carried out with the following objectives:

1. a. To determine the best condition for the longer storage of cereals.
   b. To localise the enzymes in seeds of different viability levels.
   c. To analyze the effect of GA$_3$ on the aleurone layer of seeds.
   d. To bioassay the endogenous hormonal levels during germination quantitatively.

2. a. To examine the effect of PGR-pretreatment on the storage potential of seeds.
   b. To assess the effect of PGRs on the seed viability using tetrazolium test.
   c. To assess the effects of PGRs on the protein profile of viable and semi-viable seeds by electrophoretic technique.

3. To quantify the viability of pretreated - fresh and partially aged - seeds through accelerated ageing studies.
4 To test different permutations and combinations of RH and temperature for the optimal storage.

5 To study the influence of PGRs on the partially aged seeds under the field conditions.

THE EXPERIMENTS

1a Storage Studies on Wheat, Maize and Bajra
(To determine the best condition for the longer storage of seeds)

Seeds were stored in sealed polyethylene bags of 100- and 300-gauges at ambient- (AMB : 35±8 °C), moderate- (MOD : 25±2 °C), and low-temperatures (LOW : 8±2 °C). Following studies were pursued at monthly intervals:

1 Germination Performance

MOD-stored seeds lost the viability at a faster rate followed by AMB-stored. However, LOW-stored ones remained viable even after five-year of storage. 300-gauge polythene appeared to be desirable than 100-gauge for the storage. Wheat was the best one among all the three crops. Maize lost complete viability within 28 months where as bajra remained viable up to four years. Viable seeds retained the vigour substantially. In general, vigour and viability decreased gradually with the storage period.
2 Seed Moisture Content (mc)

Moisture content was calculated on the initial-weight basis by drying the seeds at 80 °C for 72 h. It increased with the storage period irrespective of storage condition or the container. However, the magnitude of increase in mc was small in 300-gauge.

3 Enzyme Assays

Following enzyme activities were assayed in the seedlings:
* Peroxidase * Polyphenol oxidase * Protease
* Amylases (total, alpha, and beta)

The activities of peroxidase, polyphenol oxidase, and amylases declined with the advancement in the storage period, while the protease increased with the storage period.

4 Effect of growth regulators on the partially-aged seed

When the stored seed-lots lost the viability by 50-60%, they were pretreated with the PGRs (Plant Growth Regulators): gibberellic acid (GA₃ 10⁻⁵ M) and kinetin (KIN 10⁻⁵ M), keeping the distilled water (DW) as control. PGRs improved the overall performance of partially-aged seeds as compared to untreated controls (UNT). They improved the viability and vigour. GA₃ was most effective followed by KIN. Even DW was quite effective.
1b Enzyme Localisation

(To localise the enzymes in seeds of different viability levels)

The enzymes, viz, peroxidase, polyphenoloxidase, and amylase were localised in the seeds with different viability levels. The viable seeds, in general, showed higher activities in terms of colour production. The peroxidase and amylase activities can be used as a marker for the assessment of viability in cereals.

1c Studies on Aleurone Layer

(To analyze the effects of a PGR on the aleurone layer of seeds)

Viable and non viable seeds were soaked in the GA3 and DW, and sectioned transversely. The destarched sections stained with the Ninhydrin Schiff's reaction solution and were fixed and photographed. The GA3-treated seeds showed a compact and uniformly intense aleurone layer than the DW-treated. The aleurone layer in non-viable seeds appeared distorted and almost colourless or with very faint colours.

1d Endogenous Hormonal Levels during Germination

(To bioassay endogenous hormonal levels during germination quantitatively)

Endogenous GA3, KIN, Phenols and ABA were extracted and quantified at the different stages of germination, viz, 0, 24, 48, 72, 96, and 120 h, from untreated seeds. The purified extracts were paper chromatographed and bioassayed.
Endogenous levels of GA$_3$ and KIN increased up to 72 h and then slowly decreased thereafter, whereas the level of ABA decreased continuously up to 120 h. Total phenols declined constantly right from 0 h.

2a Effect of PGRs-Pretreatment on the Storage Potential
(To examine the effect of PGR-pretreatment on the storage potential of cereals)

Fresh seed-lots were subjected to the presoaking treatments of PGRs ($10^{-5}$ M) as discussed earlier in Chapter 1(A). The pretreated seeds were, then, stored in sealed 300-gauge polythene bags at different temperatures. The treated seeds maintained the high level of germinability and vigour. ABA-pretreatment reduced the loss of viability considerably.

2b Effect of PGRs on Seed Viability
(To see the effect of the PGRs on the seed viability using tetrazolium test)

The pretreated seeds with PGRs, as well as untreated (viable, semi-viable and non-viable) seeds were soaked in the tetrazolium solution and photographed. Treated seeds exhibited intense colour than the untreated ones. The viable seeds displayed maximum colouration, semi-viable the sparse colouration of moderate intensity, whereas non-viable showed no colour at all.
2c Effects of PGRs on Seed Protein Profile

(To assess the effects of PGRs on the protein profile of viable and semi-viable seeds by electrophoretic techniques)

The viable (100% germinability) and semi-viable (40-50% germinability) seeds were pretreated with GA\textsubscript{3}, KIN, and ABA (maintaining DW and UNT as controls). Electrophoretic patterns of proteins were studied using SDS-PAGE method. Three categories of seeds, viz, viable, semi-viable, and non-viable were used for isoenzyme studies of peroxidase, polyphenol oxidase, and amylases. These studies revealed fine differences among treated and untreated seeds in terms of more number of bands with high intensity. The number of isoenzyme bands gradually decreased with the increasing age.

The investigation revealed the differences between the viable and semi-viable seeds, as well as the effects of PGR-pretreatments at viable and semi-viable stages on the protein profile and isoenzyme banding.

3 Effects of PGRs on Viability of Pretreated Aged and Aged Pretreated Seeds

(To quantify the viability of pretreated - fresh and partially aged - seeds through accelerated ageing studies)

(A) The seeds were pretreated and exposed to the accelerated ageing treatment (40°C and 100% RH), to study physiological and biochemical changes.
When the germinability was reduced to 50%, untreated seeds were pretreated with PGRs and were re-exposed to the same conditions along with the untreated seeds.

**Germination Performance:** Seeds were sampled at one-day interval and germination per cent, Moisture content, Seedling length, Fresh weight, Dry weight and Vigour index has been measured.

**Leachates:** Seed samples were drawn regularly and were soaked in DW (1 g in 10 ml) for 6 h, and the following leachates were analysed quantitatively:

- Electroconductance
- Reducing sugars
- Soluble proteins
- Free amino acids
- RNA-like compounds
- Phenols

Pretreated seeds remained viable and vigorous for a longer period when compared to untreated control. The partially aged pretreated seeds brought about enhanced germinability than that of pretreated seeds. The leaching of seed solutes were found to be drastically reduced in case of the fresh-pretreated and aged-pretreated seeds. The storage potential of a given seed-lot could then be extrapolated from the results of accelerated ageing studies.

4 **Effect of Different Relative Humidities on Storage Potential**

*(To test different permutations and combinations of RH and temperature for the optimum storage)*

Seeds were sealed in polythene bags of 300-gauge and stored at ambient (AMB) and moderate (MOD) temperatures in desiccators with fixed relative humidity (RH), ie, 0%, 20%, 
40%, and 60%. Seeds stored at ambient conditions of temperature and RH were considered to be the control (UNT). Sampling was done at 3-month intervals. The following studies were undertaken:

1 Germination Performance

MOD-stored seeds lost the viability at a faster rate followed by AMB. 40%-RH-stored seeds proved to be the best in terms of extended viability. Seeds stored at other RHs displayed sub-optimum performance by losing the germinability at a faster rate. The performance of 60%-RH seeds was comparable to that of UNT. MOD-stored seeds lost viability faster than RT-stored seeds. As determined by the germination performance, the optimum RH for the storage was 40%. In wheat, UNT were the best followed by 40%-RH seeds.

2 Seed Moisture Content (mc)

In case of 40%- and 60%-RH-stored, and UNT seeds, mc increased with the storage. Whereas in 20%- and in 0%-RH-stored it decreased.

3 Enzyme assays

(a) Following enzyme activities were carried out:
* Peroxidase  * Polyphenol oxidase  * Phytase
* Amylases (total, alpha, and beta)

All the activities declined with the advancement of the storage period, except the phytase activity. Wheat and maize endosperm reflected higher amylase activities than that of bajra.
(b) Analysis of seed leachates:

Seed samples from different storage conditions were soaked in DW (1 g in 10 ml) for 6 h, and the leachates were analysed quantitatively for:

* Electroconductance
* Reducing sugars
* Total proteins
* RNA-like compounds
* Free amino acids
* Phenols

There was a gradual increase in the leakage of both, organic and inorganic, constituents of seed with the storage irrespective of storage conditions. The rate of loss of vigour and viability was promptly reflected by the pronounced leaching. Electroconductance showed a strong positive correlation with the degree of deterioration. Reducing sugars, RNA-like compounds, and amino acids also showed the similar results, whereas leaching of proteins and phenols did not offer such correlations.

4 Effect of growth regulators on the partially-aged seed

The seeds were pretreated with the plant growth regulators (PGRs): gibberellic acid (GA$_3$ 10$^{-5}$ M) and Kinetin (KIN 10$^{-5}$ M) with DW as control, after the storage of 12 months in maize, and 18 months in bajra. There was a 20-30% increase in the germinability. The pretreatments enhanced not only the viability but vigour also. GA$_3$ was very effective followed by KIN. DW appeared to be equally good.
5 Effect of PGRs on the Field Performance of Partially Aged Seeds

(To study the influence of PGRs on the partially aged seeds under the field conditions)

When the seed-lots dropped the viability up to 50%, they (partially aged seeds) were pretreated with PGRs: GA$_3$, KIN, ABA, and CCC. DW and UNT served as controls. The field performance of pretreated seeds were studied. All the pretreatments brought about significant improvements in the vegetative as well as the reproductive growth. ABA enhanced the yield attributes maximally, followed by KIN. GA$_3$ promoted the vegetative growth. DW also proved to be good.

**SALIENT FEATURES**

The following are the salient features of this study:

1a. The 300 gauge polythene bag is a better container than that of 100 gauge. The storage of seeds in sealed moisture-resistant polythene bags under low-temperature condition can prove to be superior over the ambient- and moderate-temperatures.

With the progress in the seed ageing, germination performance and enzymatic activities decrease and moisture content increases.

PGR-pretreatments improve the vigour and viability of the partially-aged seeds (germination > 50%).
1b Enzymes localisation can help in determining the different viability levels, especially amylase activity in cereals can be used as an indicator of degree of ageing.

1c GA$_3$-treatment increased the colour intensity of the aleurone layer in viable and non-viable seeds. GA$_3$ is effective in triggering the amylase activity even in the partially-aged seeds.

1d The endogenous hormonal levels (reflected by the bio-assays) of GA$_3$, KIN and ABA turned out to be favourable studies during the germination of seeds. Quantitative analyses reflect the levels of hormones during various phases of germination.

2a PGR-pretreated seeds (including DW-treated) have better storage potential. ABA pretreatment can maintain the vigour and viability for longer period during storage.

2b Tetrazolium studies can be used reasonably as a vigour test to differentiate the pretreated viable, semi-viable and non-viable seeds.

2c The electrophoretic studies of seeds can be very fruitful concerning the protein profile and the isoenzymes, as they portray substantial differences between the viable and non-viable seeds. Detailed studies may help in understanding the mechanism of PGRs at a molecular level.

3 The application of the technique of an accelerated ageing is very useful in forecasting the germination
potential of cereals within a short duration due to its relevant synchronisation with the physiological ageing.

The pretreatments to partially aged seeds rather than fresh viable is preferential. GA<sub>3</sub>- and ABA- treatments could restore the lost germinability and maintain for a longer duration.

The presoaking PGR treatments (at 50-55% germination level) could recover the rarefied germinability and vigour almost to an initial state in case of the seeds aged either naturally or artificially. The PGRs also retarded the leakage of solutes during the ageing process.

4 Storage of cereal seeds under the conditions of very high or very low relative humidities had a detrimental effect on the viability.

Enzyme activities, viz, peroxidase, polyphenol oxidase, and amylases, declined with the storage period except phytase. Leaching of the solutes upsurged with the ageing. However 40% RH could maintain the higher level of enzyme activities and less leaching for a long time. 40% RH is an optimal RH for long term storage.

5 ABA- and KIN-pretreatments to seeds proved to be the best in enhancing the vegetative and reproductive phases of growth. GA<sub>3</sub> could enhance only the vegetative growth, followed by CCC and DW showing identical results.
PGR-pretreatments to partially-aged seeds enhance the growth and yield potential under field conditions. Not only the growth promoters but growth retardants also (in lower concentration) can be very effective in maximising the yield and productivity.

It is, thus, clear from the above findings that the seeds can be stored for a longer period, and with good quality. These studies also help us in identifying the loci/sites of seed deterioration. Use of PGRs will greatly help in improving the productivity of partially aged seeds (which can be quantified for the extent of loss of viability especially in wheat and maize) or seeds of doubtful quality. These studies contribute in understanding the causes of seed deterioration and improvement in the performance of aged seeds, which is very vital for the tropical countries. It is possible not only to save the good quality seeds but also the resources of precious germplasms, especially when we are confronted with natural calamities like drought and others. Safe storage of seeds will help us in building the strong and suitable seed programmes in future.