

STORAGE POTENTIAL AND VIGOUR STUDIES ON ONION, CARROT AND LETTUCE SEEDS

A

Synopsis of the Ph.D. Thesis in Botany

By

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Research Guide

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Onion (*Allium cepa* L.), carrot (*Daucus carota* L.) and lettuce (*Lactuca sativa* L.) are major salad crops. India being one of the largest producers of onion contributes to about one-fourth of the total world production. All these three crops are useful even medicinally. However, the seeds are poor storers and cannot be stored for more than 8-10 months by the conventional methods. If the seeds are stored for more than this period the vigour decreases very drastically and seedlings perform poor.

Seed germination is the process of activation of metabolic machinery of the seed and the emergence of radical and plumule, leading to the production of seedling (Khan A A, 1977, *In: The Physiology and Biochemistry of Seed Dormancy and Germination*. Amsterdam The Netherlands; Black M, 1981, The role of endogenous hormones in germination and dormancy. *In: Control Mechanism in Seed Germination* Mayer A M (ed) pp 181-192).

Germination is the first step for the seeds to get transformed in plant. All the seeds, which are viable, possess the ability to germinate under the favourable conditions. Germination is the resumption of metabolic activity and growth by the seed tissues, involving rehydration, utilization of synthetic systems which enable the young plant to assume an autotrophic existence (Street H E and Opik H, 1970, *In: Physiology of Flowering Plants*. Edward Arnold Publishers Ltd., London).

The seed input for cultivation of any crop should be of the best quality because the seed acts as a vital link between the past accomplishments and future productivity of the crop, and the storability of a seed lot attains the status of an economically important physiological parameter.

During the phase when the original germination capacity is maintained in storage, some deterioration takes place and can be demonstrated by measurements of respiration rate, enzyme activity, leaching of solutes and seedling growth rate. In the phase of rapidly declining germination capacity, the embryos lose their ability to develop into normal seedlings and finally die. Seeds during storage proceed through a continuous process of degradation. The exact causes of loss of seed viability are still not well established. However several changes have been widely associated with seed deterioration during ageing. These changes include alterations in the enzymatic activities, rates and pathways of respiration and degradation of proteins (De Paula et al., 1996, *Physiol. Plant.*,

96: 543-550; Kalpana and Rao, 1997, *Seed Sci. & Technol.*, **25**: 271-279). In stored dry seeds oxidative stress and lipid peroxidation mediated free radicals are the probable reasons for membrane damage manifested by a breakdown of cellular permeability, which can be visualised at the ultrastructural level (Calero et al., 1981, *Crop Sci.*, **21**: 926-933; Castro and Honduvilla, 1984, *Physiol. Plant.*, **62**: 581-588).

The effect of storage deterioration on the final crop yield is the most important economic parameter. Of late the growth promoting effects of Plant Growth Regulators (PGRs) on various yield and yield contributing components and beneficial role of PGRs in modulation of seed vigour and viability have been reported (McLaren 1982, *In: Chemical Manipulation of Crop Growth and Development*, Butterworths, London, pp.4-11; Saxena and Pakeeriah, 1986, *Indian Rev. Life Sci.*, **6**: 189-214; Singh and Rathore, 1987, *Indian Agric.*, **31**: 165-169). PGRs have been reported to delay the process of aging in seeds (Petruzelli and Taranto, 1985, *Seed Sci. & Technol.*, **13**: 183-191; Persson, 1988, *Seed Sci. & Technol.*, **16(2)**: 391-404). Also it has been recently hypothesised that the embryonic axis of the deteriorating seeds remains viable long after the loss of germinability of intact seeds (Bhattacharya S and Sen Mandi S, 1995, *Indian J. Agric. Sci.*, **65(11)**: 781-784).

Hence the assessment of the storability of all these three vegetable crops under different storage conditions, and attempts to extend the seed viability and to enhance productivity of the partially aged (stored) seeds (through PGR applications) would be imperative towards the sustenance of crop productivity. Keeping in view the aforesaid facts, an indepth study of the variability in storage potential and productivity of onion, carrot and lettuce was carried out under the broad objectives listed below:

OBJECTIVES :

1. To assess the relative storage potential of onion, carrot and lettuce under two temperature conditions over a natural ageing period of two years.
2. To evaluate biochemical changes during storage deterioration in these vegetable seeds as evident by the changes in the activities of oxidative enzymes and electrophoretic profiles of different seed proteins.
- 3a. To determine the best medium for presowing and invigoration by the PGRs.

- 3b. To invigorate the partially aged seeds by PGRs and to assess the improvement at the seedling and field levels.
4. To grow *in vitro* embryos from partially aged seeds.

EXPERIMENTAL AND RESULTS

Freshly harvested seeds were hermetically sealed in glass bottles and also kept in the normal pack in which seeds are supplied by the agency. These containers were stored under:

- (a) ambient conditions (Temperature : 12-45°C, RH : 46-94%) and
 - (b) moderate temperature conditions (B.O.D. incubator - 28±2°C, RH : 36-54%).
- I. Storage deterioration at these two temperature regimes was assessed by the following parameters:
- (a) Seed moisture content;
 - (b) Germinability (per cent germination);
 - (c) Speed of germination
 - (d) Root and shoot length;
 - (e) Seedling fresh and dry weight;
 - (f) Seedling vigour index;
 - (g) Seed leachates in terms of
 - i) Electro-conductivity;
 - ii) Proteins (Lowry et al., 1951, *J. Biol. Chem.*, **193**: 265-275);
 - iii) Reducing sugars (Wharton and McCarty, 1972, *Determination of glucose*, In : Experiments and Methods in Biochemistry, MacMillan, New York, pp.196); and
 - iv) RNA like compounds (McDonald, 1955, In : Methods in Enzymology, S.P.Colowick, N.O. Kaplan (eds), Academic Press, New York) at an interval of three months.

The results depicted that the seed moisture increased with the storage period, which effected viability of the seeds, and this resulted in loss of ability of the normal germination of the seeds. Seedlings were weak after 6 months of the

storage and seedling vigour index also started declining gradually. Because of damage of the seed membrane leakage of metabolites like sugar, soluble protein, RNA, etc. increased in seed leachates with the increment in storage period.

II. Biochemical aspects of the storage deterioration included the assaying of the following enzyme activities:

- (a) Catalase (Chance and Maehly, 1955, In : *Methods in Enzymol.*, Academic Press, New York);
- (b) Peroxidase (George, 1953, *J. Biol. Chem.*, **201**: 413).
- (c) IAA oxidase (Mahadevan, 1964, In : *Modern Methods of Plant Physiol.*, Springer Verlag.);
- (d) Polyphenoloxidase (Mayer and Harel, 1979, *Phytochem.*, 18: 193);

Electrophoretic analysis (Laemmli, 1970, *Nature*, **227**: 682-685) of the total soluble proteins and major storage proteins of seed lots of different viability levels were performed to obtain the variation in protein profiles as a consequence of natural ageing.

All the above enzyme activities were slowed down with the prolonged storage period. It was very clear from the electrophoretic patterns of the proteins that there were certain proteins, which got denatured during the storage and were absent from the electrophoretic plates prepared for stored seeds. These proteins had impact on germinability of the seeds.

III. Plant Growth Regulator invigoration of partially aged seeds were carried out using two PGRs i.e. gibberellic acid (GA₃) and kinetin (KIN). Seeds were soaked in double the volume of aqueous PGR solutions (10⁻³ M to 10⁻⁷ M). The following soaking treatments were given to study the effect of PGRs alone and in combination:

- (a) Distilled water (DW);
- (b) Gibberellic acid (GA₃);
- (c) Kinetin (KIN);
- (d) Gibberellic acid + Kinetin (GA₃ + KIN).

Effect of PGRs invigoration was assessed at the seedling morphological level in the laboratory and in terms of final productivity under the field condition.

Data thus generated were statistically analysed.

Seeds soaked in DW showed a marginal increment in germination and vigour initially but after the germination declined up to 60 per cent, DW soaking was not effective. Different concentrations of PGRs were effective for different vegetable crops eg. 10^{-7} M concentration was suitable for onion and carrot while 10^{-3} M and 10^{-5} M concentrations were appropriate to improve performance of lettuce seeds. Similarly GA₃ was more effective than KIN for all the three crops while in some cases combination of GA₃ and KIN proved better.

IV. Normal plantlets from partially aged seeds were obtained employing standard tissue culture technique. Whole seeds were cultured on MS basal (Murashige and Skoog, 1962, *Physiol. Plant.*, **15**: 473-497) medium and MS basal medium supplemented with 2,4-D, BAP, KIN and NAA. Gross morphological observations were made at weekly intervals. Developing multiple shoots and plantlets were replenished/transferred to suitable media for further growth/development and root induction. Morphogenetic potential of the seeds were tested after a period of 6 months, 12 months and 24 months of storage.

MS medium supplemented with 2,4-D and BAP resulted in callus proliferation from the seeds of carrot and lettuce, while KIN and NAA were responsible for shoot proliferation or multiple shoot generation from the callus. Plain MS medium initially helped the fresh seeds to germinate but after the decline of germination below 60 per cent plain MS was not useful.

SALIENT FEATURES :

Important conclusions from the above studies are as follows :

1. Moderate temperature storage ($28\pm 2^{\circ}\text{C}$, RH: 36-54%) and sealed glass bottles are beneficial for storage of onion, carrot and lettuce seeds.
2. Among all the three vegetable crops lettuce was the best storer while onion was the poorest storer.
3. Storage deterioration is evident from a decreasing trend exhibited by the oxidative enzyme activities and an increase in the metabolites in the seed leachates.

4. PGRs invigorated seeds performed better at seedling level as well as under field conditions indicating that post storage seed priming treatment with PGRs is beneficial in reviving the performance of partially aged seeds.
5. Embryos can be rescued from partially aged seeds and multiple plantlets can be raised through tissue culture.

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