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Chapter VI

SUMMARY AND CONCLUSIONS

Presented in this chapter are the summary and conclusions of the investigations carried out on the seeds of soyabean (Glycine max L. (Merr.) cv. Bragg) stored at 10°C, 30°C and ambient temperature (27.6 ± 10°C) highlighting the influence and various physiological, biochemical and histological parameters associated with it. The results of field studies are also summarised.

The seed occupies a unique position in plant sciences. In that it is of great economic importance as an important source of our food supply. One of the most important factors contributing to maximum crop production per unit of land is the use of good seed. Seeds should have high germination capacity and vigour and should be able to perform both against pest, diseases and competition of weeds. Planning for seed storage can be done effectively if sufficient information is available on the relative storability of various cultivars under different agro-climatic conditions of our country.

After the success of green revolution, now the attention has been shifted towards pulse and oilseeds. Soyabean is receiving special attention in our country because of its high protein (40-47 %) and oil content (20-22 %). Hence present

studies were undertaken with the following objectives:

I Physiological and biochemical changes associated with seed deterioration during 24 month storage at 10°C, 30°C and ambient temperatures, respectively.

II Accelerated ageing studies at mid storage including estimation of leachates.

III Tetrazolium studies for predicting the quality of stored seeds and diagnostic patterns of seed disturbances.

IV Histochemical localization of metabolites in viable, semi-viable and non-viable seeds at 24 and 120 h of germination.

V Influence of gibberellic acid (GA_3) on the performance of field crops.

6.1 Biochemical Changes During Seed Deterioration

Soyabean cv. Bragg seeds with 6-7 per cent moisture were stored in polythene bags (300 gauge) at 10° and 30°C temperature and a lot for comparison was stored in cloths bags under ambient conditions (27.6 ± 10°C). The samples were taken out every four months and physical, physiological and biochemical estimations were carried out after growing the seeds in DW and GA_3 (10 ppm). The following conclusions are drawn:

6.1.1 Percent germination rate, fresh wt, dry wt and seedling length were higher at 10° followed by 30°C as compared to ambient conditions.

6.1.2 Catalase, peroxidase, polyphenol and IAA oxidase activities showed a decline with the degree of deterioration, the declining being faster under ambient conditions followed by 30°C and 10°C.

6.1.3 Carbohydrate, protein and nucleic acids exhibited well defined behaviour with the loss of viability in soyabean.

6.2 Accelerated Ageing Studies

These studies were undertaken at mid storage by exposing soyabean at 41 ± 1°C & 100 % relative humidity for 24, 48, 72 and 96 h. Seeds after equilibrisation were germinated in distilled water (DW) and gibberellic acid (GA_3^{10ppm}) and physiological studies were undertaken and the leachates were analysed for various physiological and biochemical parameters.

6.2.1 Percent germination and seed moisture, seedling lengths, fresh wt and dry wt and per cent moisture as well as dehydrogenase activity showed a positive correlation with the loss of viability.

6.2.2 Generally, the leakage was higher under ambient conditions followed by 30°C as compared to 10°C. There was increase in the

electro-conductance values, peroxidase, sugars, invertase, amylase, tyrosine, tryptophan, oricinal +ve and diphenylamine +ve compounds indicating greater damage to the cell membrane due to artificial accelerating ageing. These results go hand in hand in hand with the data on biochemical changes associated with seed deterioration.

6.2.3 Incorporation of gibberellic acid and kinetin through acetone is very promising in improving percent germination of soyabean seeds stored for longer duration (8 months) and gibberellic acid surpassed kinetin in its better performance. Growth hormones help in minimising the leakage of metabolites and enzymes indicating the possible repair of the membrances.

6.3 Tetrazolium studies

Viability status, disturbance and weaknesses associated with deterioration were assessed by carrying out tetrazolium localization in viable ($10^{\circ}C$); semi-viable ($30^{\circ}C$) and non-viable (ambient) stored seeds. The following are the conclusions:

6.3.1 The tetrazolium provided a visual picture of the quality of soyabean under storage at various temperatures. Thus, the presence, frequency and sequence of all causes of disturbance in ageing seeds could be followed till the seed became dead.

6.3.2 The germination percentage of soyabean seed stored at different temperatures and period of time can be predicted with

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the following equations:

$Y = 12.841 + 0.857\ x$ for 10°C (a)

$Y = 0.408 + 1.024\ x$ for 30°C (b)

$Y = 15.185 + 0.690\ x$ for ambient (c)

where,

Y = Predicted germination percentage

x = Percent viability as indicated by tetrazolium test.

6.3.3 Alternatively, a stain scoring out of a maximum of 10 pluses (4 for radicle, 3 for plumule and 3 for cotyledon) can be used for predicting germination percentage as under:

$Y = 3.80 + 8.57\ x$ for 10°C (a)

$Y = 7.90 + 7.97\ x$ for 30°C (b)

$Y = 7.97 + 7.93\ x$ for ambient temperature (c)

where,

Y = Predicted germination percentage

x = TTC stain scoring out of 10 pluses

6.3.4 Diagnostic patterns of both internal and external damages to the seed have been identified. Differences between diagnostic TTC staining symptoms of initial and advanced causes

of disturbances and their performance in germination test could be understood with great precision with the help of TTC staining.

6.3.5 Based on this study, a set of 30 different staining patterns have been identified, developed and photographed as compared to the established 15 patterns usually used by the seed certification laboratory in India. The information on additional patterns will be very useful to seed technologists in future to compare and interpret their results on soyabeen and their crop seeds.

6.4 Histochemical and Histological Studies

In order to assess the concentration and localization of polysaccharides, proteins and nucleic acids, histochemical and histological studies were undertaken at the end of 16 months of storage at 10°, 30° and ambient temperatures.

6.4.1 Water damage caused lysigenous cavities and invited fungal infection which caused the loss of starch grains even in the radicle of viable seedlings.

6.4.2 The formation of deep PAS +ve channel (devoid of starch grain) in the intercellular spaces was very clear in semi-viable seeds showing detriment and such features were not observed in viable seedlings (seeds stored at 10°C). These channels provided the passage for leakage of biological compounds.

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6.4.3 Absence of starch in different tissues of the non-viable seedlings was verified with I_2-KI test.

6.4.4 Concentration of insoluble polysaccharides per unit area was maximum in plumule of 10°C seedlings followed by 30°C and ambient temperatures.

6.4.5 Total proteins were highest in plumule followed by radicle at 24 h germination in 10°, 30° and ambient condition, which becomes more or less constant at 120 h.

6.4.6 Average number of parameters associated with seed deterioration such as complete disappearance of total protein, frequent occurrence of channels, lysis of cell walls, complete loss of discreteness, decrease in size of various growth organs, loss of metabolites, etc. are clearly presented here.

6.4.7 Metacutinization in the roots of the non-viable seeds was clearly evident.

6.4.8 In the non-viable seedling RNA concentrations were very low in all the parts and at all the germination stages.

6.4.9 The histological changes were evident in non-viable seedlings with pronounced necrotic areas.

6.5 Field Studies

In order to investigate the effect of storage temperatures and pre-soaking treatments of aged seeds on field emergence,

plant growth and yields, the field experiments were conducted. The following conclusions are drawn:

6.5.1 It confirms the well-established fact that the reduction in viability below 50 per cent does affect the final yield, significantly.

6.5.2 There appears to be a relationship between the laboratory germination per cent to the field emergence of the aged seeds. The reduction in field emergence, as compared to the laboratory test may vary from 10-20 per cent if the seeds are stored from one harvesting season to the next planting season. Accordingly, the seed rate may be adjusted/enhanced over the recommended rate.

6.5.3 Pre-soaking of seed in GA_3 (100 ppm) does help in field emergence of aged seeds. There were an increase of 4 and 18 per cent in seed emergence over laboratory germination tests on the 8-month old seeds stored at 30°C and ambient conditions, respectively.

6.5.4 Pre-soaking of soyabean seeds in GA_3-100 ppm increased the seed yield by 50 to 65 per cent for the aged seeds. Similar findings are available for other crops by other researchers of this laboratory. Hence, extensive trials are required to commercialize these techniques and its economic studied.

6.5.5 Finally, there is co-relation between the storage temperature, laboratory germination test and field emergence,

Plant growth and yield are directly affected by these parameters. The study confirms that percentage seed emergence is an excellent index of the loss of yield potential of the surviving seeds under agricultural conditions.

6.6 Concluding Remarks

6.6.1 Finally, it is concluded that the soyabean seed can be stored safely in polythene bags of above 300 gauge at 10°C without much loss of viability as revealed by the physiological, biochemical, tetrazolium and histochemical studies. Soyabean seed (like most crop seeds) gained moisture appreciably when stored under ambient conditions in cloth bags.

6.6.2 Accelerating studies are quite useful in predicting the storage potential of seeds in the shortest possible time.

6.6.3 The use of bio-regulants, especially gibberellic acid (GA_3) offers an immense potential practical application in agriculture when partially aged seeds can perform significantly as compared to old seeds.

6.6.4 Valuable new informations on additional patterns of tetrazolium localization will help in explaining many variations in correlating tetrazolium test with seedling emergence and which hitherto could not be possibly explained.

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6.6.5 These studies have provided an integrated picture embracing many disciplines like embryology, anatomy, physiology and biochemistry of soyabean seeds. Finally these studies were confirmed under field conditions.