Chapter - VIII

INVIGORATION OF NON-VIABLE SEEDS OF *Brassica campestris* L.cv.B-54 DUE TO TREATMENT WITH VITAMINS FOR THEIR COMMERCIAL UTILISATION IN THE PLAINS OF DARJEELING DISTRICT, WEST BENGAL.

INTRODUCTION

Rapid deterioration leading to non viability of seeds is a serious problem in tropical and sub- tropical countries like India and specially in North Bengal, where temperature and high relative humidity greatly accelerate seed ageing phenomenon resulting in their deterioration. Seeds of *Brassica campestris*- B-54 in particular are sensitive to these adverse climatic conditions and often becomes subjected to rapid deterioration under ambient storage environment. If the same cultivar is introduced this may create problems for plant growers of the region. It is a common experience that huge amount of *Brassica* seeds are being lost each year during their storage.

Presently, seed invigoration for improved performance is a subject attracting a lot of attention of seed scientists. The term invigoration is however, broad and rather vague and would probably remain so in the absence of a proper concept of seed vigour. Perry (1978) defines vigour as "the sum total of all those properties of the seed which determine the potential level of activity of the seed during germination and seedling emergence". It would therefore appear that any treatment which brings about an overall improvement of seed performance may be considered as invigoration treatment. Thus beneficial post harvest treatments with chemicals to the stored seed may bring about qualilative improvement in seed performance.

Non viability of seed may be considered as ultimate complete loss of orgainisation and function. It is not simply a running down of the life process but is highly ordered and programmed process or series of processes. Once the seed attains non viability it is very difficult to reverse the situation and like all other living mechanism seed also should have some thresh hold storage life within which there may be a possibility of qualitative improvement of seed performance. According to Ovcharov (1977) vitamin content of seeds drops due to prolonged storage, Skrabka (1965) increased germination of wheat seeds stored for nine years with the treatment of a mixture of chemicals containing ascorbic acid. No systematic study on the invigouration of *Brassica* seeds stored for longer period has yet been taken into consideration. Here an attempt has been made to study the effect of different vitamins on germination behaviour of seeds stored for different duration of time in *Brassica campestris*. B- 54 to determine thresh hold storage life of the same so that storage seeds of the plant could be utilised for commercial purpose in the region.

MATERIALS AND METHODS

MATERIALS

Brassica campestris L.B-54 was cultivated in the experimental plot in North Bengal University campus and seeds were collected from the mature fruit of the plant for their utilisation during investigation.

METHODS

GERMINATION OF SEEDS

For studying the effect of vitamins on germination, seeds were presoaked in respective chemicals for 18 hours and were placed in petridishes containing cotton pad soaked with chemical solution. These were kept in a temperature and light controlled BOD incubator at 27±1°C under white light supplied by incandescent lamp (1200 Lux). Distilled water was added subsequently every alternate day to maintain constant moisture level in each set. Seeds were considered to have been germinated when emerging radical was easily observed and final observation on percentage of germination was recorded after five days from initiation of the experiment. Each experiment included three replicates each containing 100 seeds. Seeds presoaked with only distilled water was treated as control.

PREPARATION OF CHEMICAL SOLUTION

Stock solution (1M) for each of vitamins was prepared. From the stock solution different dilutions of 10^{-3}, 10^{-5} and 10^{-7}M were produced and utilised during seed germination study. The following vitamins were used during seed germination experiment.

Riboflavin, Ascorbic acid, Pyridoxine hydrochloride, Nicotinic acid, Pantothanic acid, Thiamine hydrochloride.

EFFECT OF STORAGE CONDITION ON AND VIABILITY OF SEEDS

Seeds were stored in clothed bag kept in the laboratory in dry condition. Time to time seeds were checked to free them from insect or fungal attack. This was continued for six years. To study the loss of total viability, the stored seeds were examined for their germination behaviour in distilled water in each year upto the duration of total six years of storage.

TTC TEST

To perform TTC test (2,3,5- triphenyl tetrazolium chloride) 100 seeds were soaked for 24 hours in deionised distilled water, seed coat was then removed and immersed in 1% TTC solution. The rose red formazon was extracted following the method of Powell and Mathew (1977) after taking to embryonal axes with three replicates in 10ml. of 95% ethanol (v/v) at 80°C for five minutes. After cooling the absorbance of the extract was measured in a Spectrophotometer (C.Z. Instruments) at 520 nm.

During separation of tissue dissecting microscope was used. Rating of seeds on the basis of variation of TTC staining was done visually and catagorised as follows :

I. Whole seeds (colytedons and embroyo) deeply red coloured.

II. Embryo deeply red coloured and cotyledons with deep patches of colouration.

III. Embryo and cotyledons faintly red coloured.

IV. No red colouration.

RESULTS AND DISCUSSION

Seed deterioration is a natural catabolic process which terminates the life span of seed, resulting in loss of viability. The process may be accelerated externally by some pathogenic attack or by adverse environmental condition otherwise the deteriorative events may follow their normal course culminating the production of non-viable seeds. This inevitable deterioration of seed is a matter of serious concern to the seed physiologists and various stratagies are being developed to prolong the storage potentially of seeds.

Reduced seed germinability as well as slower rate of germination are considered to be important visible criteria for the evaluation of poor seed vigour (Anderson, 1970; Abdul-Baki and Anderson, 1972). But with the help of seed germinability it is very difficult to distinghish between non- viability and dormancy of seed. According to Mayer and Poljakoff- Mayber (1978) all histochemical methods devised to test viability are based on the activity of certain oxidizing enzyme and the best correlation has been found to be the activity of enzyme reacting with redox dyes such as tetrazolium. Viability of stored seeds of

B.campestris B-54 has been tested with tetrazolium. Colour (Table-33) and rating categories have been observed (Table-34). Table-33 shows that the optical density of formazon colour complex in the cotyledons and embryo gradually decreased along with decreased percentage of seed germination in *Brassica campestris* B- 54 due to prolonged storage. Seeds of B. campestris B-54 have been noted to attain non- viability after 6 years. Table-33 shows assessment of viability in *B. campestris* B- 54 seeds subjected to different period of storage by tetrazolium colour reaction. From Table-33 it appears that all the seeds of *B. campestris* B-54 were viable upto storage period of one year but it enters non-viability in the second year of storage and afterwards. The viability decreased in the subsequent years of storage and nearly 60% of seeds were observed to become non- viable when stored for 5 years.

Vitamins were observed to show invigoration of germination of stored seeds, Table-35 shows that there was no significant effect of vitamins on the freshly collected seeds. But nicotinic acid and ascorbic acid triggred upto 89% in both the cases as compared to control showing 82% of seed germination (Table-33) when seed stored for one year. Riboflavin, and Pantothanic acid had some effect of invigouration on the same type of seeds.

Table-37 shows overall stimulatory effect of all vitamins on the germination of seed stored for two years. Out of all vitamins. Riboflavin, Ascorbic acid, Pyridoxine hydrochloride, Nicotinic acid have significant stimulation showing 74, 74, 70 and 75 percent of seed germination respectively at 10^{-5} (M) concentration for all vitamins as compared to control (58%). Similar is the result with the seeds stored for three years (Table-38). In this connection Riboflavin, 10^{-4}(M) Ascorbic acid (10^{-7}M), Pyridoxine hydrochloride (10^{-5}M) and Nicotinic acid (10^{-5}M) show maximum of 52, 58, 50 and 55 percent of seed germination respectively as compared to control showing only 35% of seed germination (Table-38). Invigoration effect has been observed to decrease in the seeds stored for four years (Table-39). Though significant stimulation of seed germination has occurred due to Riboflavin, Ascorbic acid, Pyridoxine hydrochloride and Nicotinic acid treatment maximum of invigoration has been observed due to ascorbic acid showing 34% of seed germination at the concentration of 10^{-5} M as compared to control (12%).

Table-40 shows that seeds stored for five years had significant deterioration as only 1% of seed germination occurs in control. Though significiant invigoration

was observed due to treatment with Ascorbic acid (10^{-7} M) showing 7% of seed germination, the over all low percentage of seed germination will not be profitable enough for their commercial use. Thus it may be suggested that seed of *Brassica campestris* B- 54 stored maximum for three to four years will be suitable for their commercial use after being pretreated with some of the vitamins such as ascorbic acid and nicotinic acid.

Seed of different plants contain different quantities of vitamins. The quantity of these vitamins in seeds of plants from one and the same variety grown under different conditions also differs (Ovcharov; 1977). Therefore, it is understandable that under certain conditions seeds accumulate the quantity of vitamins necessary for their germination while under other conditions the vitamin content is insufficient.

It was pointed out earlier that non- germinated seeds of various plants had significantly low quantities of vitamin and amide of nicotinic acid occurred within the seed and according to Ovcharov (1977) vitamin content of seeds drops due to prolonged storage. He also suggested the activity of enzymes in plant parts increased under the influence of vitamins and vitality of essential compounds were like wise stimulated. Due to treatment of Nicotinic acid quantity of RNA, DNA, basic and non- basic protein increased significantly.

Sulakadze et al, (1955) observed significantly increased percentage of germination by the treatment of seed with nicotinic acid and thiamine. Similar observation was recorded by Zakhar Yants et al (1966) that seeds of maize at different physiological stages of ripeness responded differently to an additional supply of biotin. Wheat seeds showed different germination capacities in presence of 0.01% solution of ascorbic acid and a 0.001% solution of glutamic acid led to intensive synthesis of auxins (Skrabka, 1965). The germination of wheat seeds stored for 9 years was increased after soaking them in copper sulphate in a solution of glutamic acid or in a mixture of glutamic acid, ascorbic acid and copper sulphate for 14 hrs. Smith et al (1959) showed that vitamin B_6 performed amination and assimilation of metabolites and Pyridoxine participated in the biosynthesis of the y-amino buteric acid. Billi (1954) suggest that pyrodoxine participates in the biosynthesis of the y-amino butyric acid that forms at the time of seed germination. In presence of pyridoxne phosphate manganese peroxide catalyzed oxidative decarboxylation of tryptophan with the formation

of indole acetamide which ultimately converted to indole acetic acid. With a deficiency in this vitamin many aspects of amino acid synthesis are disturbed. With the enrichment of seeds and seedlings with this vitamin the metabolic process is normalized.

Table - 33

Percentage of seed germination during storage and TTC test on the viability of seeds in *Brassica campestris* B-54.

Years of collection of seed for storage	Percentage of seeds germination	Duration of storage (in year)	Rating category	OD value of Formazon colour comples
1996	93	0	I	0.31
1995	82	1	I	0.25
1994	58	2	II	0.19
1993	35	3	II	0.16
1992	12	4	III	0.10
1991	1	5	III	0.02
1990	0	6	IV	0.00

Rating category

I. Whole seed (cotyledon and embryo) deeply red coloured.

II. Embryo deeply red coloured and cotyledon with deep patches of colouration.

III. Embryo and cotyledons faintly red coloured.

IV. No red colouration.

Table - 34

Assessment of viability in *Brassica campestris* B-54 seeds during different period of storage by Tetrazolium colour reaction.

Storage Period (Yrs)	Total nos.of seeds	Percentage of seed responding to colour after 24 hrs. under different rating categories.		
	I	II	III	IV
0	100	100	-	-
1	100	85	15	-
2	100	60	15	5
20				
3	100	40	20	15
25				
4	100	10	25	20
45				
5	100	5	10	25
60				
6	100	-	-	-

Rating category

I. Whole seed (cotyledon and embryo) deeply red coloured.

II. Embryo deeply red coloured and cotyledons with deep patches of colouration.

III. Embryo and cotyledons faintly red coloured.

IV. No red colouraion.

Table - 35

Effect of vitamins on germination of seed collected immediately after harvest in *Brassica campestris* B-54.

Treatment	Conc. (M)	Percentage of germination.
Riboflavin	10^{-3}	90
	10^{-5}	95
	10^{-7}	94
Ascorbic acid	10^{-3}	92
	10^{-5}	94
	10^{-7}	95
Pyridoxine hydrochloride	10^{-3}	92
	10^{-5}	94
	10^{-7}	93
Nicotinic acid	10^{-3}	90
	10^{-5}	92
	10^{-7}	90
Pantothanic acid	10^{-3}	85
	10^{-5}	91
	10^{-7}	90
Thiamine hydrochloride	10^{-3}	92
	10^{-5}	90
	10^{-7}	91
Control		93

Mean :	91.67%
S.E :	0.57%
C.D at 5% level :	1.21%
C.D at 1% level :	1.66%

Table - 36

Effect of vitamins on germination of seed stored for one year in *Brassica campestris* B-54.

Treatment	Conc. (M)	Percentage of germination.
Riboflavin	10^{-3}	81
	10^{-5}	86
	10^{-7}	84
Ascorbic acid	10^{-3}	82
	10^{-5}	89
	10^{-7}	87
Pyridoxine hydrochloride	10^{-3}	80
	10^{-5}	84
	10^{-7}	81
Nicotinic acid	10^{-3}	80
	10^{-5}	89
	10^{-7}	81
Pantothanic acid	10^{-3}	69
	10^{-5}	87
	10^{-7}	75
	10^{-3}	78
Thiamine hydrochloride	10^{-5}	85
	10^{-7}	73
Control		82
	Mean :	81.7%
	S.E :	1.29%
	C.D at 5% level :	2.71%
	C.D at 1% level :	3.73%

Table - 37

Effect of vitamins on germination of seed stored for two years in *Brassica campestris* B-54.

Treatment	Conc. (M)	Percentage of germination.
Riboflavin	10^{-3}	20
	10^{-5}	74
	10^{-7}	72
Ascorbic acid	10^{-3}	68
	10^{-5}	74
	10^{-7}	71
Pyridoxine hydrochloride	10^{-3}	65
	10^{-5}	70
	10^{-7}	62
Nicotinic acid	10^{-3}	63
	10^{-5}	75
	10^{-7}	62
Pantothanic acid	10^{-3}	48
	10^{-5}	60
	10^{-7}	54
Thiamine hydrochloride	10^{-3}	49
	10^{-5}	59
	10^{-7}	50
Control		58
	Mean :	60.9%
	S.E :	3.17%
	C.D at 5% level :	6.69%
	C.D at 1% level:	9.19%

Table - 38

Effect of vitamins on germination of seed stored for three years in *Brassica campestris* B-54.

Treatment	Conc. (M)	Percentage of germination.
Riboflavin	10^{-3}	46
	10^{-5}	52
	10^{-7}	49
Ascorbic acid	10^{-3}	52
	10^{-5}	56
	10^{-7}	58
Pyridoxine hydrochloride	10^{-3}	37
	10^{-5}	50
	10^{-7}	49
Nicotinic acid	10^{-3}	32
	10^{-5}	55
	10^{-7}	30
Pantothanic acid	10^{-3}	28
	10^{-5}	35
	10^{-7}	30
Thiamine hydrochloride	10^{-3}	31
	10^{-5}	35
	10^{-7}	28
Control		35
	Mean :	41.8%
	S.E :	2.57%
	C.D at 5% level :	5.42%
	C.D at 1% level :	7.45%

Table - 39

Effect of vitamins on germination of seed stored for four years in *Brassica campestris* B-54.

Treatment	Conc. (M)	Percentage of germination.
Riboflavin	10^{-3}	15
	10^{-5}	17
	10^{-7}	25
Ascorbic acid	10^{-3}	32
	10^{-5}	34
	10^{-7}	32
Pyridoxine hydrochloride	10^{-3}	19
	10^{-5}	21
	10^{-7}	15
Nicotinic acid	10^{-3}	12
	10^{-5}	15
	10^{-7}	12
Pantothanic acid	10^{-3}	8
	10^{-5}	10
	10^{-7}	9
Thiamine hydrochloride	10^{-3}	11
	10^{-5}	8
	10^{-7}	9
Control		12
	Mean :	16.9%
	S.E :	2.03%
	C.D at 5% level :	4.28
	C.D at 1% level :	5.88%

Table - 40

Effect of vitamins on germination of seed stored for five years in *Brassica campestris* B-54.

Treatment	Conc. (M)	Percentage of germination.
Riboflavin	10^{-3}	2
	10^{-5}	3
	10^{-7}	1
Ascorbic acid	10^{-3}	6
	10^{-5}	5
	10^{-7}	7
Pyridoxine hydrochloride	10^{-3}	2
	10^{-5}	2
	10^{-7}	1
Nicotinic acid	10^{-3}	3
	10^{-5}	1
	10^{-7}	1
Pantothanic acid	10^{-3}	1
	10^{-5}	1
	10^{-7}	1
Thiamine hydrochloride	10^{-3}	1
	10^{-5}	1
	10^{-7}	1
Control		1
	Mean :	2.22%
	S.E :	0.45%
	C.D at 5% level :	0.94%
	C.D at 1% level :	1.30%

Thus there is a possibiltiy that seeds of *Brassica campestris* B-54 during storage suffers from a deficiency in some vitamins and the timely supply of the requisite vitamins to such seeds intensifies metabolism and improves their germination.

Brassica seeds are generally classified as orthodox type (Roberts etal 1973). The orthodox seeds can be safely dried to a low moisture content in the air dry range, the storability of such seeds improves with the lowering of seed moisture.

A question of great fundamental interest to the germination and storage, physiologists are concerned with the nature and extent of influence of the events occurring during dry storage of seed. The integrity of nucleic acids DNA and RNA during the air dry state is of special interest in this connection. Studies on nucelic acids of dry dicotyeledonous seeds have shown that total DNA and RNA may not undergo any change but some species of RNA are more stable than others (Roberts *et. al.*, 1973) however, when storage conditions are not ideal, fragmentation of DNA into smaller units may take place.

Osborne (1982) detected the presence of RNAase that would degrade ribosomal RNA in embryo extracts of non- viable seeds. Roberts and Osborne (1973) showed an increase in DNAase activity during loss of vitality.

We know that in a well preserved seed many bio chemical reaction start soon after imbibition of water. This means that the cells of viable seeds retain the required organelles and molecules in a potentially functional state during the phase of dessication and which is not available at the nonviable state of seeds. An impairment of membrane functions would have far reaching consequences as not only the plasma membrane but the orderly arrangement of membrane bound proteins would also be adversely affected in undesirable degradation reactions. The cumulative effect of all these cause catastrophic events which would culminate in the loss of vigour and viability of the seed.

It is not possible to acheive an absolute control of the deteriorative senescence of the seed and to stop the ageing process for once and all. In modern gene banks longevity for thousands of years is contemplated. Robert etal (1973) have provided data to show that barley seeds stored at 5% moisture and -20^{0}C would go down from 95 to 90% viability for a longer period.

A number of authors applied hydration and dehydration treatment of stored seeds of *Brassica* and which could minimize their physiological deterioration during subsequent humid conditions. They have also noted a significantly lower lipid peroxidation in mustard seeds treated with various ageing stresses.

As lipid peroxidation may involve free radical chain reaction (Harman and Maltick, 1976;) it may be argued that physico- chemical process of hydration and dehydration treatment counteract free radical interaction.

There is no doubt that we are in lack of a clear understanding of the mode of action storage of seeds and their invigoration treatments perhaps, a formidable bottle neck in our agriculture. The limited availability of quality seeds, could be partly over come by adopting the right kind of seed invigoration treatment.

Study on vitamin treatment in connection with invigoratin of non- viable seeds of *Brassica* and specially *Brassica campestris* B- 54 has not been done earlier. It is now being recommended for its commercial use in Darjeeling condition. Modern technology for storage of mustard seeds is very necessary. It is expected that vitamin treatment as represented in this part of work will be suitable for making availability of quality of seeds of *B.campestris* B-54 out of useless non- viable seeds produced during their storage. This is very much effective to seeds stored atleast upto three to four years in Darjeeling conditions.

SUMMARY

Decline in percentage of germination of seeds of *B. campestris* B-54 has been observed during their storage.

Seed reaches non viability after six years of storage in laboratory condition.

Rating category of relative stages of nonviability of stored seeds. has been verified with the help of TTC test and degree of redness of seeds caused by formazon due to triphenyl tetrazolium chloride treatment has been observed.

All the seeds have been observed to remain completely viable during storage of one year but starts entering into nonviable condition in the second year of storage and after wards 60 percent of non viable seeds has been observed during storage for 5 years. Several vitamins like Riboflavin, Ascorbic acid Pyridoxine hydrochloride, Nicotinic acid have significant stimulation showing 74, 74, 50 and 75 percent respectively at 10^{-5} (M) of concentration for all vitamines as compared to control (58%) seeds stored for two years.

In connection with the seeds. stored for three years, Riboflavin (10^{-4}M) ascorbic acid (10^{-7}M) Pyridoxine, hydrochloride (10^{-5}M) and Nicofinic acid (10^{-5}M) show maximum of 52, 58, 50 and 55 percent of seed germination respectively as compared to control showing only 35% of seed germination.

Seeds stored for five years show significant deterionation as only 1 percent of seed germination occurs in control, though in vigoration has been observed due to treatment of Ascorbic acid (10^{-7}M) Nicotinic acid (10^{-5}M) showing maximum of 7 percent of germinations for both the treatments.

It is suggested that seeds of *B.campestris* B-54 stored maximum of 3 to 4 years are suitable for their commercial use after being pretreated with suitable vitamines.