DISCUSSION

DISCUSSION

Deterioration of seeds under ambient storage conditions or seed senescence is an internal programmed phenomenon which leads to loss of vigour followed by loss of viability and consequent death and decay of seeds. Depending upon the genetic make-up of seed species, the process of seed deterioration under storage is quickened or delayed determining the life span of a specific seed type. Like plant senescence (Leopold and Kriedemann, 1975; Thimann, 1980; Biswas and Ghosh, 1999) the process of seed senescence is complicated and the pattern varies with the broad classes of seeds like orthodox and recalcitrant seeds and even with the seed species of different plant taxa. Accordingly, the mechanism of seed senescence is no less complex than the complicated senescence processes of higher plants.

Maintenance of vigour and viability of seeds is an important problem in agriculture and horticulture. The two environmental factors, i.e. temperature and relative humidity (RH), have profound influence on seed health under storage (Copeland and McDonald, 1995; Desai *et al.*, 1997). In recent years, some effective physical and chemical manipulative techniques have been developed by seed technologists to get rid of such climatic as well as biotic (Halloin, 1986; Agarwal and Sinclair, 1997) hazards which are conducive to earlier deterioration of stored seeds. In the literature dealing with seed viability, there are some reports that hydration-dehydration treatment as well as treatment of seeds with chemials of diverse nature (salts, phenols, organic acids, antioxidants, essential oils, plant growth regulators etc.) can favourably influence the viability status of seeds (Basu *et al*, 1979; Savino *et al*, 1979; Pathak and Basu, 1980; Chhetri *et al.*, 1993; Basu, 1994; Rai *et al.*, 1995; Bhattacharjee *et al.*, 1999).

The experiments of the present investigation ~~was~~ were performed with the seeds of four different cultivars with a view to evaluating their viability status under unfavourable storage environment by analysing a number of putative and reliable biochemical parameters. But emphasis was laid on prolongation of seed viability under storage by using a pretreating chemical Na-dikegulac, which seems to be promising in this regard in a few recent studies (Bhattacharjee and Gupta, 1985; Bhattacharjee and Choudhuri, 1986;

Bhattacharjee *et al*, 1986a; Chhetri *et al.*, 1993; Rai *et al.*, 1995; Bhattacharjee *et al.*, 1999). Results obtained in this investigation were critically discussed on the basis of numerous information available to date, and the prospect of Na-dikegulac as a beneficial pretreating chemical on the maintenance of storage potential of seeds is focussed.

The present study shows that high RH treatment rapidly accelerated the ageing of gram, soybean, sunflower and safflower seeds even under the short-term accelerated ageing period for three weeks only and viability status was found to be appreciably low. Under long-term accelerated ageing for 112 days seed viability was drastically impaired. Data revealed that in short-term ageing leakage of soluble carbohydrates (Table 1) and amino acids (Table 2) from the forced-aged seeds progressively increased with the ageing duration, and this increase of leaky substances was considerably checked by seed pretreatment with Na-dikegulac. The same trend of result was recorded in long-term accelerated ageing but here the ageing effect was much more injurious (Figs. 2 and 3). However, a significant alleviation of the injurious effect was noted in seeds which underwent pretreatment with Na-dikegulac.

The results are indicative of the fact that accelerated ageing caused to damage seed membrane which consequently resulted in higher leakage of soluble substances, and the pretreating chemcial alleviated this deleterious effect to a considerable extent. The membrane is the most important site of a seed which appears to be attacked first by any accelerated ageing treatment (Ching and Schoolcraft, 1968; Harman and Granett, 1972) and arguably any chemical purported to have an effect on seed viability must influence membrane integrity. The proposal that a decrease in membrane integrity and occurrence of membrane lesions might play a significant role in the deterioration of seeds has been supported by work on solute leaching accompanying a fall in germinability and viability (Thomas, 1960; Nutile, 1964; Harman and Granett, 1972; Powell and Matthews, 1977; Halder *et al*, 1983; Bewley, 1986). Under accelerated ageing condition, along with the fall of seed germination, phospholipid and phosphatidyl choline – the important components of membrane also decline (Halder *et al*, 1983; Francis and Coolbear, 1984; Basu, 1994; Desai *et al.*, 1997) leading to loss of membrane integrity. This loss is reflected in enhanced leaching of organic and inorganic metabolites from seeds into the imbibing medium (Abdul-Baki and Anderson, 1973; Halder, 1981; Kole and Gupta, 1982). Zilkah and Gressel (1979, 1980) reported that dikegulac-induced changes of membrane permeability has been due primarily to changes in the plasmalemma. Increased permeability of cellular membranes (presumably the plasmalemma) of wheat and pea seeds due to fungal infection or accelerated ageing treatment was shown by Anderson (1970) and Harman and Granett (1972). There are also reports that ability of the seeds to reorganize its membrane rapidly as the desiccated tissue rehydrate is a crucial to successful germination. Much evidence has been put forward to suggest that membrane status within the germinating embryo is an important factor in deterioration (Desai and Tappel, 1963; Harman and Mattick, 1976; Copeland and McDonald, 1995).

The supporting references presented on membrane integrity and loss of quality of seeds, thus, indicate that the forced ageing-induced membrane damage of gram, soybean, sunflower and safflower seeds, as observed in this investigation, might possibly be a reason for their rapid loss of viability during storage. Significantly lesser leaching of soluble carbohydrates and amino acids from the chemical-pretreated seeds is suggestive of the fact that Na-dikegulac rendered the seeds tolerant against storage deterioration under unfavourable environment by retaining the integrity of seed membrane.

During accelerated ageing period, changes of leachable carbohydrates and amino acids were associated with a proportional shift in metabolism within seed kernels of the four crops species. Efficacy of Na-dikegulac on the maintenance of seed health can also be supported from the changes of a number of biochemical parameters analysed here which are considered as reliable indices of seed vigour. Data showed that in short-term accelerated ageing level of protein (Table 3), RNA (Table 4) and insoluble carbohydrate (Table 6) gradually declined in control samples with ageing duration and this declining trend was considerably slowed down by the pretreating chemical. Again, in case of long-term accelerated ageing a drastic reduction of protein (Fig. 4), RNA (Fig. 5) and insoluble carbohydrate (Fig. 7) was noted. Here also, the pretreating chemical efficiently relieved the deleterious effect of forced ageing treatment. Na-dikegulac also arrested the alarming rise of internal soluble carbohydrate level as observed in both short-term (Table 5) and long-term (Fig. 6) experiments.

The above results, therefore, point out that although deterioration is a common phenomenon both in treated and in control samples of the crop seeds, the catabolic processes within the treated seeds remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environments. Available reports show that during seed ageing loss of some vital cellular components occurr (Abdul-Baki and Anderson, 1972; Kole and Gupta, 1982). Increase of soluble substances such as sugars and amino acids (Ching and Schoolcraft, 1968; Bhattacharjee, 1984; Chhetri *et al.*, 1993) and decrease of nucleic acids (Bhattacharjee and Gupta, 1985; Cherry and Skadsen, 1986; Anderson and Gupta, 1986) during the process of seed deterioration has also been documented. The results obtained, thus, are in conformity with the reported observations.

That the pretreating chemical is efficient in substantial alleviation of the damaging effect of accelerated ageing can be supported from the analyses of total dehydrogenase activity of seed kernels as well as from percent TTC-stained seeds. Data showed that both dehydrogenase activity and percent TTC-stained seeds progressively declined in short-term (Tables 7 and 8) ageing experiment and abrupt reduction of the enzyme activity and TTC stainability of all the seed species was noted in case of long-term (Figs. 8 and 9) ageing experiments. The pretreating chemical partially averted the adverse effects in both the cases.

Dehydrogenase activity is generally used as a reliable index for the evaluation of seed viability (Abdul-Baki and Anderson, 1972; Copeland and McDonald, 1995). There are also reports that as seeds age, they lose vigour which is evaluated by counting percentage TTC-stained seeds and/or by observing the pattern of TTC-staining which appears as deep red colour or as irregular red patches on the seeds, depending on their viability status (Halder, 1981). Data, thus, point out that in spite of experiencing accelerated ageing treatment, the chemical-pretreated seeds got hardened and retained higher vigour than the control ones, and such hardening is effected at the metabolic level.

The efficacy of Na-dikegulac on maintaining storage potential of seeds can be substantiated from the comprehensive work done with the sunflower seeds which underwent accelerated ageing for 10, 20, 30 and 40 days. Data revealed that percentage seed germination and field emergence (Table 9), speed of seed germination (Table 10) as well as root length, shoot-length and seedling dry weight (Table 11) were adversely affected in control samples under accelerated ageing condition, and the inhibitory effects were reversed to some extent by the pretreating chemical. Reduced seed germinability and slower rate of germination are considered to be the important visible criteria for the evaluation of poor seed vigour (Anderson, 1970) and such reduced vigour is reflected in seedling establishment (Bhattacharjee, 1984; Roberts, 1986). The results of this investigation is thus in conformity with the reported observations. As Na-dikegulac ameliorated the harmful effects of accelerated ageing on seed germination behaviour and seedling growth the chemcial seems to harden the seeds under unfavourable storage situation.

Accelerated ageing-induced damage of metabolic status of seeds as evidenced from reduction of DNA and RNA (Table 12) levels, accumulation of soluble carbohydrate and free amino acids (Table 14), subdued activities of dehydrogenase and catalase enzymes (Table 14), enhanced activities of amylase and protease (Table 15) enzymes in seed cotyledons and relieving action of Na-dikegulac pretreatment are indicative of storage potentiation property of this chemical. Results clearly point out that although seed deterioration is a common phenomenon both in the chemical pretreated and control samples, the catabolic processes within treated seeds remained somewhat subdued thereby rendering the seeds tolerant against unfavourable storage environment. Loss of some vital cellular components during seed ageing is well-documented (Abdul-Baki and Anderson, 1972; Kole and Gupta, 1982). Increase of soluble substances such as sugars and amino acids during the process of seed deterioration has also been shown (Ching and Schoolcraft, 1968; Bhattacharjee, 1984). Perl *et al.* (1978) in their experiment with *Sorghum* seeds reported that increase of protease activity occurs under accelerated ageing with concomitant reduction of some anabolic enzymes. The results of this investigation thus corroborates the reported observations of some other workers.

Accelerated ageing treatment for 30 days impaired field performance of sunflower plants as evident from the reduction of some growth parameters like plant height and stem circumference (Table 16), leaf area and plant dry weight (Table 17) and that of some biochemical parameters like chlorophyll and protein (Table 18), RNA (Table 19), activities of catalase and superoxide dismutase enzymes (Table 20) as well as enhancement of soluble carbohydrate level (Table 19) and activities of IAA-oxidase and protease enzymes. The chemical-induced alleviation of the deleterious effects of accelerated ageing on the overall growth and metabolism of sunflower plants thus indicates the potential retention action of Na-dikegulac in sunflower plants. The ageing-induced adverse effects on overall growth and metabolism of sunflower plant was reflected in the inception of important developmental stages of sunflower plant and in yield attributes. Data revealed that inception of radicle emergence, leaf emergence, head initiation, ray floret opening, 50% floret opening, head yellowing and harvest (Table 22) were significantly delayed in plants raised from the accelerated aged control seed samples, and Na-dikegulac partially relieved the delaying action on the inception of the above mentioned developmental stages. Again, reduced field performance of plants were associated with concomitant reduction of yield attributes leading to impairment of final seed yield (Table 23) of the plant which were developed from the forced aged control seeds. Here also, Na-dikegulac showed a promising role as the adverse effects on plant development and crop yield were alleviated to a considerable extent.

That the pretreating chemical helped to maintain storage potential of sunflower seeds can also be corroborated from the experiment done by imposing water-stress treatment on seedlings, raised from accelerated aged and nonaged seeds. The pretreating chemical efficiently nullified to a significant extent, the ageing-induced as well as water stress-induced reduction of seed germinability (percentage and T50 of germination, Table 24), height and dry matter content (Table 25), chlorophyll and protein content (Table 26) as well as activities of catalase and superoxide dismutase enzymes (Table 27) of seedlings.

Adaptive responses of plants towards environmental stress are indicative of their high vigour and these are reflected in metabolism through gene expression (Hochachka and Somero, 1973). That the chemicals are efficient in substantial alleviation of the damaging effects of water deficit stress can be supported from the analyses of seed germination behaviour, seedling growth and metabolism. Data showed that percentage of seed germination and T50 hours were positively affected in the chemical treated seed samples. Results, therefore, pointed out that in spite of experiencing accelerated ageing (by high RH) followed by water-stress treatment (by PEG 6000), the chemical pretreated seed lots retained high vigour and produced healthier seedlings than the control ones. Chlorophyll and protein are the vital substances maintaining the normal functional life of plants. Again higher activity of the H_2O_2 scavenger enzyme catalase (Fridovich, 1976) was also shown in plants having higher potential and maintaining a vigourous growth (Sarkar and Choudhuri, 1980). Comparatively higher levels of these biochemical parameters in the chemical pretreated samples prove the invigourating action of the chemical.

Thus, it can be concluded that Na-dikegulac is not only effective in enhancing storage potential of sunflower seeds but equally efficient in improving seedling vigour as evident from the result which clearly shows that the chemical treated seeds were less sensitive to water deficit stress than the control ones.

As regards the mechanism of dikegulac-induced maintenance of seed vigour, nothing is known till date and no clear picture is emerged from the present study also. This much can be interpreted from this investigatioin that Na-dikegulac helps in substantial maintenance of seed health of all the deteriorating seed species under adverse storage environment by slowing down the metabolic processes and by retaining membrane integrity. But this interpretation is absolutely tentative, because so many factors are closely associated with seed deterioration process and controlling of these factors positively influence seed health under storage. One such important factor is storage microorganisms. There are reports that under storage condition, fungal attack is checked or their incidence is reduced in seeds by some growth retardant treatments (Sinha and Wood, 1964; Tahori *et al*, 1965; El-Fouly and Jung, 1966). As fungicidal or fungistatic effect of this less explored retardant has not yet been analysed, perhaps it is not wise to put forward any consolidated proposition on dikegulac-induced regulation of seed viability at the present state of our knowledge and information available till date. It is not unlikely that the pretreating growth retardant Na-dikegulac might have rendered seeds less susceptible to fungal damage, thereby influencing the maintenance of seed vigour at least for a certain period of storage.

Again Na-dikegulac-induced regulation of free redicals and consequent improvement of seed health under storage may not be ruled out. The basis of free-radical pathology is the presence of unpaired electrons on the radicals. Free radicals participate, chiefly in the form of activated O_2 species such as superoxide (O_2) or H_2O_2, in several electron transfer reactions of normal cell metabolism and are usually controlled by appropriate protective mechanism, such as by the activities of superoxide dismutase, catalase and peroxidases (Halliwell, 1978, Elstner, 1982). Of the possible biophysical and biochemical causes of seed deterioration, free-radical damage leading to a disruption of the functions of cellular membrane (Tappel, 1973) assumes significance. Pammenter *et al.* (1974) found that provision of a source of free electrons significantly extended seed viability. Basu and Pal (1979) suggested that quenching of naturally produced free radicals would be of advantage in controlling seed senescence. Bhattacharjee and Choudhuri (1986) observed higher activity of H_2O_2-scavenger enzymes (Fridovich, 1976) catalase and also superoxide dismutase in Na-dikegulac-pretreated jute seeds under accelerated ageing condition. Thus, in the present study also, it may be quite likely that Na-dikegulac helped to retain the vital cellular components and strengthened the defence mechanism by stimulating the activities of free radical scavenging enzymes, thereby rendering seeds tolerant against unfavourable storage environment.

Whatever might be the exact mechanism of Na-dikegulac-induced regulation of seed vigour and viability, there is least doubt that this chemical can enhance storage potential of seeds and maintain seed health under storage for longer duration which would be evident from the results of present study. In fact, it is clear from the present investigation that Na-dikegulac alleviated the accelerated ageing-induced deleterious effects on seed germination behaviour, seed metabolism, seedling growth and metabolism, plant growth and metabolism at three development stages as well as overcome, at least partially, the ageing-induced and water stress-induced strong impairment of the overall performance of seed germination behaviour, seedling growth and metabolism.

In future researches, if this beneficial action of Na-dikegulac is established from a wide range of crop seeds, the practice of conventional methods of seed storing may be suitably substituted by this chemical. Now, it is a challenge to the modern researchers working in this field to devise newer and better strategies for effective exploitation of Na-dikegulac for retention of seed vigour and viability. We look forward to answering to this problem in the coming decade.