Discussion
Midstorage hydration-dehydration treatment of stored wheat seeds, with or without chemicals, has been found to be very effective in controlling seed deterioration during subsequent storage. It has, however, been shown that the physiological age of the seed at the time of treatment is a crucial factor in determining the effectiveness of the treatment. In all the cultivars of wheat, treatment of 4-6-month-old seeds proved to be most effective in controlling the loss of vigour and viability. Soaking (followed by drying) of harvest-fresh seeds adversely affected their storability. This confirms the earlier reports from the present laboratory on the beneficial effects of mid-storage hydration-dehydration treatments on the maintenance of vigour and viability of seeds of a number of crops (Basu et al., 1974; Basu and Dasgupta, 1974; Basu et al., 1975).

Seed hydration could be accomplished by soaking, dipping for a very short period and moisture equilibration with a saturated atmosphere for a prolonged period. Of the different methods of hydration, soaking and dipping are very simple; in the former, seeds were soaked in water for 2 hours but in the latter the seeds were dipped for 2 minutes and then taken out and kept covered for another 2-3 hours before drying back. Moisture equilibration with a saturated atmosphere (followed by drying)
proved very effective but it is difficult to practise especially when large seed lots are concerned.

The studies on the germinability of seeds immediately after treatment and after subjecting the seeds to various ageing conditions clearly reveal that the effects of treatments on immediate (pre-storage) germinability are not conspicuous, only after sufficient physiological deterioration upon ageing, the treatment differences show up. The usual manifestations of the treatment effects were greater germination percentage, reduced percentage of morphologically abnormal seedlings and greater uniformity in growth and development. These are indeed the normal manifestations of high seed vigour (Delouche et al., 1967; Heydecker, 1972; Delouche, 1969). In physiological and biochemical studies, greater membrane integrity and increased activity of dehydrogenase enzyme were noted in treated seeds. The productivity of the crops raised from the treated seeds was also significantly greater than that from the untreated control.

The beneficial effects of mid-storage seed treatment on field performance and productivity were noted in all the cultivars of wheat which were studied viz. Sonalika, Janak, UP 262 and WL 711.
Studies in the present laboratory with the treated (hydration-dehydration treatment) and untreated seeds of a range of crop seeds (Dasgupta et al., 1976; Dasgupta, 1978; Panjabi et al., 1979; Basu and Dhar, 1979; Panjabi and Basu, 1979; Mitra and Basu, 1979; Basu et al., 1979; Kundu and Basu, 1981) as well as the present findings on wheat confirm that a 15-20% difference in germination percentage (difference between control and treatment) is significantly reflected in yield.

In peas, beans and barley, Abdulla and Roberts (1969) noted a significant loss of final yield only when the seed viability was reduced to about 50%. The observations of Harrison (1966) on lettuce would, however, indicate a considerable loss of final yield even with a small loss of viability. Similar views have been expressed by several other workers (Grabe, 1964; Delouche et al., 1967; Heydecker, 1972). According to Delouche et al. (1967), one of the early effects of seed deterioration is decline in yield. The germination capacity is considered to be a late effect of deterioration and only when deterioration has progressed considerably a germination test may be able to detect the same.
In the present investigation, the results of the field experiments with wheat seeds showed a significant increase in yield and other yield attributes like plant population per unit area, number of shoots per unit area, 1000 grain-weight and number of grains per ear in plants raised from the treated seeds. All the cultivars of wheat responded almost similarly showing significant improvement in field performance and productivity with hydration-dehydration treatments.

Both soaking-drying and dipping-drying treatments showed significantly higher grain yield than control. Water alone was very effective but chemicals such as sodium phosphate (dibasic, $10^{-4}$ M) showed significant improvement over water. Studies on seed treatment with micronutrients, however, gave no additional effects of the salts over water.

The hydration-dehydration treatments with or without seed protectants (dry dressing with fungicides and light spraying with the insecticide, malathion) showed significant improvement in grain yield over untreated control. Thus the major effect was due to hydration (and then dehydration). Pre-sowing seed treatment in wheat following the present methodology has been found to be either ineffective or only marginally effective, as in such treatments the field stand and other yield attributes were
not affected. The midstorage treatments which reduced physiological deterioration, showed a better field stand and improved yield attributes. The significant increase in yield was not merely due to an increased plant population because of higher viability but also to an effective maintenance of the intrinsic vigour of the seed. Even a very stable character such as 1000-grain weight was significantly higher in the crop raised from the treated seeds. This is of special significance in view of the fact that the control plants suffered less interplant competition because of lower population density.

That the treatments very effectively retained seed vigour is further simplified by the significant alleviation of salinity stress by the treated seeds.

It may be pointed out here that, in general, the population density in our field experiments has been comparatively low in spite of using the recommended seed rate (100 kg per hectare). The effect of planting density on various yield components of wheat has been critically discussed by Saini and coworkers (Saini and Wanda, 1979; Saini et al; 1980) and perhaps in our experiments further increase in plant density would have increased the yield. The lower plant density may be attributable to the relatively high temperatures during sowing and in the initial
stages of crop growth in this part of the country and possibly higher commercial planting density for such situations should be recommended in future. Nevertheless, in this study, major emphasis has been given to seed treatment effects and it may be assumed that even with higher densities, the beneficial effects of the treatments would be sustained. The improved field performance of the seeds of the mid-term hydration-dehydration treatments was related to the maintenance of vigour and viability and could therefore be looked upon as the consequences of a reduced physiological deterioration of seeds during storage.

Mode of action of seed treatments

In a series of papers, Henckel and coworkers (Henckel, 1968, 1972; Henckel and Ivanitskaya, 1967; Henckel et al., 1968) have shown the beneficial effects of presowing wetting-drying treatments on the performance of plants under stress conditions. A comprehensive account of the research work carried out in Soviet Russia and elsewhere on the various aspects of seed treatment has been given by Orcharov (1977). May et al. (1962) report that in many cases, the effects of seed treatments on crop stand and productivity is rather inconsistent; marginal or non-significant.

According to Henckel (1968, 1972), the beneficial effects of presowing treatments were associated with the formation of more high energy
compounds, increased DNA and RNA in the growing points, less active ribonuclease activity, active protein synthesis, higher mitochondrial activity and better preservation of cellular ultrastructure with allied sequential changes in the elasticity and viscosity of the protoplasm.

The improvement caused by pre-sowing wetting-drying treatment in carrot was attributed by Austin et al. (1969) to pre-enlargement of embryo and advancement of germination. Similar observations have also been made by Hutchinson (1969) and Hegarty (1970) in interpreting the improved field emergence of one variety of carrot and two varieties of sweet corn. However, the aforesaid hardening treatments are also effective in other species, where the embryo is already fully developed. Therefore, the effects cannot be explained in terms of embryo enlargement only.

Biochemical evidences in favour of germination advancement by hydration-dehydration pre-treatments have been greatly emphasized by Osborne and coworkers (Osborne et al., 1974; Sen and Osborne, 1974). Berrie and Drennan (1971) showed that biochemical changes during germination advancement hydration-dehydration treatment of tomato and oat seeds enhanced protease
activity. According to Sen and Osborne (1974), the hydration-dehydration pretreatment of rye embryos initially enhanced the ability of the embryo to synthesize protein and RNA compared to the untreated controls. Heydecker (1974) was also of the opinion that germination advancement taking place in osmotic treatments would give a quicker germination so as to tide over inimical soil and environmental conditions.

Mode of action of mid-storage hydration-dehydration treatments

The present method of seed treatment is different from the above noted pre-sowing wetting-drying treatments. The treatment has to be given after the seeds are stored for several months and there should be a time gap between treatment and sowing. The effect is primarily on the storability of the seed. As such, unless the seeds are stored, the effects would not be discernible.

We may, however, examine here several hypotheses for interpreting the beneficial effects of the mid-storage seed treatments taking into consideration the observations made in this investigation and the results of other relevant studies on seed storage and seed treatments.

During the initial phase of germination, the treated seeds always showed faster germination and increased root and shoot growth. The
differences were however, non-significant after germination for 120 hours. Further, in harvest-fresh seeds also a similar advancement of germination was noted, although in those seeds the treatments were ineffective. If germination advancement would have been the reason of the beneficial effect of the treatments, fresh seed treatment should have given similar beneficial effects on the maintenance of vigour and viability as noted in stored seeds.

Germination advancement Basu et al. (1979) reported that the germination advancement is not an important reason of the beneficial effect of soaking-drying treatments on viability of dormant lettuce seed.

Differential moisture absorption by seeds from a humid-atmosphere could be an important factor in seed deterioration. But no significant difference in moisture uptake by hydrated-dehydrated and control seed was noted during ageing under different conditions.

Further, the beneficial effect of treatments were noted even in storage under 36% RH and 40°C, an environment from which no absorption of moisture by seeds took place. Non-effectiveness of fresh seed treatment would also eliminate differential moisture absorption as a factor in the maintenance of vigour and viability.
by hydration-dehydration treatments.

In earlier reports from this laboratory, it was indicated that leaching out of toxic substances from the seeds extended their storage life (Basu et al., 1974; Basu and Dasgupta, 1974). A positive relation between accumulation of growth inhibitory substances and loss of viability has been demonstrated by several workers (Dey and Sirkar, 1968; Dey et al., 1967; Sircar and Biswas, 1960; Sircar and Dey, 1967). The effectiveness of seed treatment by dipping-drying and especially by moisture equilibration-drying, in which cases seed hydration was unaccompanied by leaching, would suggest that the beneficial effects of mid-storage treatments cannot be attributed to the leaching out of inhibitors from the seeds.

A possible antifungal effect of the soaking-drying treatments would also not explain the beneficial effect. Even when the seeds were aged at 36% RH and 40°C, the treatments showed highly significant effects. This ageing condition is considered unsuitable for the growth of storage microflora (Christensen, 1972). In the present studies, in which seed protectants were used either in combination with the hydration-dehydration treatments or singly, the major effect was attributable to hydration-
Role of dehydration. The fungicidal treatments alone were mostly ineffective indicating that, in the ambient condition, storage fungi did not play a significant role in seed deterioration; the reasons of loss of viability were primarily physiological in nature.

Villiers (1974) and Villiers and Edgcumbe (1975) observed that in fully imbibed seeds, repair of vital bioorganelles would take place and as a result storage life of the seed would be extended. In dry-stored seeds, in the absence of a repair mechanism, the damage to macromolecules would accumulate. Villiers (1975) has shown that when dry-stored lettuce seeds were fully imbibed not only was the senescence stopped but a definite reversal of the damage to the chromosomes and membranes took place. This has been considered to be due to the activity of repair mechanism in the hydrated seeds rather than a mere stabilization of the macromolecular structures by hydration.

The beneficial effects of hydration-dehydration treatment of stored wheat seeds have been shown to be primarily prophylactic in nature rather than curative. The hydration-dehydration treatments did not show any significant improvement of seed germinability immediately after treatment. But after
ageing, highly significant differences were noted between control and treated seeds.

Another observation which is worth mentioning here is that there was little change in membrane functions by hydration-dehydration treatment. Immediately after moisture equilibration of the stored seed with a saturated atmosphere, the electrical conductance of the seed-steep water was reduced but on drying back to the original moisture content, the electrical conductivity of the leachate became similar to that in control seeds. This would indicate the temporary and reservible nature of the changes in membranes during hydration. A repair of damage inflicted on the biomembranes would cause a permanent change leading to a noticeable lowering of the electrical conductance immediately after treatment.

The oxidative deterioration of polyunsaturated lipids in cellular components involving the reactions of free radical intermediates as the most primary reason of senescence of the cell as reported by Tappel (1973). The basis of free radical pathology is the presence of unpaired electrons on the radicals which confer on them the power to react very energetically and initiate non-specific hydrogen abstraction and chain propagation reactions. Provision of a source of free electrons enabled Pammenter et al. (1974) to control significantly the physiological deterioration of maize seeds.
The effect was apparently brought about by pairing of the unpaired electrons of free radicals with the electrons by cathode tubes.

Irradiation has a damaging effect on the germinability of seed and to a certain extent, the radiation-induced deterioration mimics natural ageing in that it reduces seedling growth and increases chromosomal aberration. In radiobiological experiments with seeds, hydration-dehydration treatments considerably wiped out the radiation-induced free radical signals (Cook, 1963; Conger and Randolph, 1968; Haber and Randolph, 1967). Perhaps in hydrated seeds, the free radicals became mobile enough to recombine into harmless non-radical products (Ehrenberg, 1961). The hydration-dehydration treatments which extended viability showed significant protection against X-irradiation damage, thereby indicating a relation between free radical pathology and seed deterioration (Dasgupta et al., 1977). The physiochemical treatments possibly minimized deterioration by controlling free radical reactions (Basu and Dasgupta, 1978).

As regards the role of chemicals in wheat seed deterioration, compared to water their effects were very small and in many instances statistically not significant. The chemicals in present study were selected on the basis of previous studies in this laboratory with seeds of wheat and other crop plants (Basu and Dasgupta, 1974, 1978; Dasgupta...
et al., 1979; Mitra and Basu, 1979; Pathak and Basu, 1980) and on the basis of their possible effectiveness in controlling free radical reactions as antioxidants, antioxidant synergists and radioprotective agents (Nickerson, 1967; Heckly and Dinnick, 1967; Dertinger and Jung, 1970; Slater, 1972; Demopoulos, 1973; Milvy, 1973). Pathak (1980) introduced a range of free radical controlling agents into mustard seed by the dry permeation technique employing acetone as the solvent and could significantly extend the storage life of seeds. In her studies, chemicals, which are generally known to accentuate free radical reactions, accelerated the loss of seed viability.

The involvement of the free radicals in the destruction of the lipoprotein cell membrane is well documented (Barber and Bernheim, 1967; Desai and Tappel, 1963). In this context, Rudrapal and Basu (1979) showed a small but consistent reduction in lipid peroxidation in hydrated-dehydrated wheat seeds. A decrease in lipid peroxidation was observed in mustard seeds which were pretreated with free radical controlling agents (Pathak, 1980). In the present study also, the different hydration-dehydration treatments showed a reduction in lipid peroxidation of wheat seeds subjected to accelerated ageing after treatment.
The results therefore, suggest that the maintenance of seed germinability by mid-storage treatments could be attributed at least in part to a counteraction of lipid peroxidation reactions.

Seed treatment: as a practical proposition

The hydration-dehydration method of seed treatment described in the present thesis has been developed with small seed lots. For larger seed lots, as handled by seed merchants, suitable technological adaptation would be necessary which does not seem to be difficult. Recently, in Tamil Nadu, ten metric tonnes of hybrid bazra seeds have been successfully treated (3 hours soaking in 10^{-4}M disodium hydrogen phosphate followed by drying back in the sun) by this method (Dharmalingam et al., 1980). Field experiments on a wide scale in different localities are, however, necessary in order to make fruitful recommendations to the wheat growers for accepting the methodology. For the average farmer, handling smaller seed lots, the method should be readily acceptable and the Directorate of Agriculture, Government of West Bengal has taken up pilot experiments on seed treatment with the view to recommending the method to the farmers of the State. The results so far obtained by the Directorate have indicated a definite possibility of the method being recommended officially for the preservation of wheat seeds in this State in the near future.