

STRATEGIES TO IMPROVE THE SEED QUALITY AND STORABILITY OF SOYBEAN - A REVIEW

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ABSTRACT

Production of high quality soybean seed which could retain its viability through a storage season is a major challenge in most areas of the humid tropics and sub-tropics. The rapid seed deterioration of soybean is thought to be due to lipid peroxidation. Subsequently resulting in loss of seed quality and viability. The seed invigoration of soybean could be a tool in improving the quality of soybean seed during storage. Soybean seed reaches its maximum potential for germination and vigour at physiological maturity and starts deteriorating on plant itself if harvesting is delayed (field weathering). Mechanical damage is another major factor responsible for deterioration in seed quality during post harvest processing and storage. Care must be taken during harvesting, threshing, processing, packing, transporting and storage to reduce the rate of deterioration in seed quality of soybean.

Seed deterioration leads to reduction in quality, performance and stand establishment. It is difficult to quantify the economic losses caused by the poor seed performance. The seed invigoration provides an important tool for improving seed vigour, improved germinability, greater storability and better field performance. Similarly proper environmental conditions and post harvest handling may prevent the soybean seed quality deterioration.

Strategies for improving storability :

A) Invigoration

Invigoration is a simple technique of hydration and dehydration of seed as mid-term storage treatment. Heydecker (1972) believes that seed could be invigorated. He pointed out that during first 24 h from the beginning of imbibition, seed go through a series of irreversible steps towards visible germination yet can be dried back without suffering. The leaching of toxic metabolites from the seed may promote subsequent germinability. Most studies suggest that the hydration phase cause activation of essential germination and repair enzymes. These remain semi-activated following dry back and are quickly reactivated on imbibition culminating in more rapid and uniform germination.

Damage to cellular membrane and other essential organelles by autoxidation has been put forward as a possible reason for seed senescence. For superiority in germination percentage from invigorated seeds than the untreated seeds, two possible mechanism had been put forth.

1 The involvement of the cellular repair system in correcting age induced biochemical lesion during seed hydration (Villiers and Edgcumbe, 1975, Osbame, 1982, Burgass and Powell, 1984).

2 Counteraction of free radical and lipid peroxidation reaction by hydration-dehydration (invigoration) treatment [Basu (1976), Basu and Dasgupta (1978), Berjak (1978), Rudrapal and Basu (1979), Dey and Basu (1989), Chouduri and Basu (1988)].

Villiers and Edgcumbe (1975) showed not only maintenance of initial viability but also a distinct reversal of age induced chromosomal observation. According to them, in air dry storage (5-20 % mc) or in seed equilibrated with a water saturated atmosphere such repairs are not possible. Villiers (1975) suggested that the beneficial effects of full imbibitions is due to the activity of repair mechanism in moist stored seed rather than the stabilization of the macromolecular structure

by hydration. The fact that the treatment effects were largely prophylactic would indicate that some factor responsible for further damage were eliminated by the physico-chemical treatments. Considerable evidences exist that repair of DNA protein membrane and enzymes occurs during imbibition. Increasing seed moisture content hasten the repair process (Ward and Powel, 1983). Oxygen also increases the repairs of high moisture (27 to 44 %) of seed. In this connection, the role of free radical assumes significance. In well hydrated tissue free radical absorbent (eg. tocopherol) or scavenging reaction limit the extent of undersirable oxidation. An important free radical scavenger is the enzyme superoxide dismutase, it converts the superoxide radical to H_2O_2 which in turn is removed by catalase. The free radicals could be quenched immediately after their generation, much of the future damage due to their chain propagation could be prevented. Thus, Parmenter *et al.* (1974) successfully extended seed viability by providing a source of free electron which would pair with the unpaired electron of free radical. Choudhari and Basu (1988) demonstrated that hydration dehydration (H-D) treatment effectively slowed physiological deterioration under natural ageing conditions. This improved storability that was associated with greater dehydrogenase activity and appreciably lower peroxide formation in cells. This might be reason for increased percentage of viability as tested by TZ test. Saha *et al.* (1990) showed that priming caused increased amylase and dehydrogenase activity in aged soybean seed compared to unprimed seeds while lowering lipid peroxidation. The invigoration treatment may act as a preventive measure against ageing. Lower lipid peroxidation and lower free fatty acid formation in treated seeds over untreated suggest that invigoration treatment may act atleast partly scavenging free radical formed during dry storage.

Basu (1976) reported that the loss of viability of seeds of several cereals, pulses, oil seeds and vegetables under ambient and accelerated ageing conditions could be significantly slowed down by soaking the seeds half way during storage with water or dilute solutions (10^{-5} - 10^{-2} M) of a range of chemicals for 2-6 h followed by drying back to original weight. Water soaking and drying itself has been found to be effective in majority of seeds investigated. Soaking in double the volume of water gave better results in majority of the materials. Treatments of fresh seeds or very old seeds were less effective than treatment of stored seeds (after 4-6 months of normal storage) of good germinability. The soaking and drying treatment improve the immediate germinability of seeds but the beneficial effects were greatly magnified upon storage. The extended seed viability may be possibly due to reduced free radical damage to the cellular components.

Savino *et al.* (1979) reported two conclusions from their results that presoaking had an improving influence upon the vigour of low quality seeds before ageing. Treated seeds maintained viability and vigour for a longer time than the control. Dey and Mukherjee (1986) stored the seeds of mustard, soybean and maize under different temperature and humidity conditions and reported that hydration-dehydration treatments both short and long duration effectively extended the storage life of mustard and maize seed and maintained high vigour potential. They observed greater activity of dehydrogenase, peroxidase and lowering of free fatty acids formation, lipase activity and lipid peroxidation are suggested to have contributed to the higher germinability of treated seeds over untreated control. They concluded that invigoration treatment brought about significant improvement in the storage life and vigour of maize and mustard seed. This improvement

was found to be related to an enhanced activity of dehydrogenase and peroxidase with simultaneous reduction in lipolytic activity and fat breakdown products. In soybean hydration-dehydration treatment failed to show any noticeable improvement possibly due to soaking injury caused by hydration. Saha *et al.* (1990) reported that moisture equilibrium soaking drying (ME-S-D) and moist sand conditioning soaking drying (MSC-S-D) treatments of stored seeds of soybean cultivar 'Soyamax' greatly reduced the loss of vigour and viability under accelerated as well as natural ageing conditions. Their post ageing physiological and biochemical studies showed increased amylase and dehydrogenase activities and greater membrane integrity coupled with significantly low lipid peroxidation in the treated seeds than the control. They suggested that the beneficial hydration dehydration treatments ME-S-D and MSC-S-D improved seed vigour and viability of soybean at least in part by counteracting lipid peroxidation.

Mukhopadhyay *et al.* (1997) stated that treatment of high vigour (fresh harvested) *P. vulgaris* and soybean seed with some powdered plant material and pharmaceutical product controlled the loss of vigour and viability of seed stored under ambient conditions. The beneficial effects of the treatments on storability were associated with reduction of volatile gaseous products from the treated seeds. Singh and Dadlani (1998) undertook investigation to study the effect of mid-storage treatment on germination and vigour of four cv. of soybean *viz.*, PK 327, PK 472, MACS 13 and JS 71-05 and found that dry dressing with bleaching powder (BLP) was most effective in enhancing germination and seedling vigour in all cultivars followed by MED (moisture equilibration drying), whereas BHT (butylated hydroxy toluene) treatment resulted in slight inhibition. Germination and vigour

declined significantly after six month of storage in all cultivars irrespective of treatment except in JS 71-05 where seed treated with BLP retained 100 % germination as against 94 % in untreated control and 92 % in MED. In case of MACS 13, MED was most beneficial though this cultivar exhibited poorest germination and vigour than other cultivars. Rao *et al.* (1999) obtained significant yield advantage by invigoration treatment with 0.5 % potassium nitrate to the seeds.

The germination percentage, vigour, dry matter content of invigorated soybean seed was significantly higher than the untreated seeds during storage irrespective of variety, threshing and processing method and storage containers. The germination percentage above MSCS of invigorated seeds was maintained more by 30 days than that of untreated seeds. The increase in storability could be ascribed to the prevention of lipid peroxidation by invigoration or leaching of toxic metabolites during invigoration. The mode of action of the viability maintenance of invigoration treatment is not yet clear. The root shoot (RS) length of invigorated seed of soybean was higher than the RS length of untreated soybean seed during storage (Shelar, 2002). The beneficial effects of invigoration treatment to stored seeds on maintenance of vigour and viability of a number of non leguminous crop plants (Basu 1976, Basu and Dasgupta, 1978, Rudrapal and Basu 1979, Mandal and Basu, 1983) and modified invigoration treatment in soybean (Saha and Basu, 1982, 1984) have been reported.

Ashraf and Bray (1993) found that 70-80 % DNA replicated during priming was plastids and mitochondrial number increased during priming. Mitochondria provide ATP necessary for rapid seedling growth during germination. This may be one of the key factors associated with improved seedling growth following invigoration. Priming also reverses

seed deterioration and their beneficial effects generally occur in the meristematic axis or the radical tip. Fu *et al.* (1988) showed that the embryonic axes of peanut seeds were most sensitive to deterioration. Osmoconditioning with 20-25 % PEG for 48 h increased germination metabolism in aged axes more than in cotyledones. Dell Aquila and Toranto (1986) demonstrated that primed embryos of aged wheat seeds had a faster resumption of cell division and DNA synthesis. Priming influences conductivity results. The lower electrical conductivity of the invigorated seed than the untreated soybean seed during storage may be cause of repairs mechanism operated in the seed during imbibition. Imbibition by viable seed is accompanied by a rapid but transient efflux of inorganic and organic compounds through the plasmalemma and tonoplast membranes and into the surrounding solution. Moreover, membrane integrity is incomplete for at least several minutes after water uptake. But the situation is reversed with time the membrane either physically reverted to their most stable configuration or else being repaired by some still unidentified enzymic mechanism. In low or non-viable seeds such repair mechanism might be absent or insufficient or the membrane might be so badly damaged that repair is impossible (Bewley and Black 1978). Argerich and Bradford (1989) reported that primed tomato seeds did not leak as much as non primed seeds. This was attributed to the washing off of external solutes during priming but is likely to be the result of restriction of membrane structure during the priming period. Lower electrical conductivity following priming were also reported for egg plant and radish (Rudrapal and Nakamura, 1988) and onion seeds (Chaudhari and Basu 1988).

The leaching of sugars were also reported to be less from the invigorated seed as compared to leaching of sugars from

untreated seeds (Shelar, 2002). This could be attributed to the loss of membrane integrity in deteriorated seed. Upon imbibition, more substances leak into the medium from such deteriorated seed than from viable ones. Excess leakage of sugars may represent loss of respirable substrate. McDonald (1999) showed that soybean seeds establish a resistance to seed leakage at seed moisture content above 24%. Natural ageing of French bean seed stored upto for 4 years induced membrane disruption and leakage of ultra violet absorbing substances which were ameliorated by priming (Pandey 1988a, 1988b).

B) Environmental performance :

Growing soybean in *Kharif* season is a common practice. However, the seed produced during *Kharif* has to store for at least 5-6 months. The quality may deteriorate faster during storage. If the quality of seed produced in summer or other season is good, it may give the alternate source of good quality seed. Tang *et al.* (1994) in their trial with 7 soybean cultivars sown in spring and autumn, observed that pre-harvest seed deterioration was about twice as compared to post-harvest deterioration. They reported that the cause of this deterioration was physiological changes due to high temperature and high moisture conditions. They observed that the effects of pathogen infection were small and the extent of seed deterioration varied with cultivar and differed between spring and autumn seeds. Reddy *et al.* (1995) reported that soybean grown in two different seasons *viz.* *Kharif* and *rabi* and threshed by three methods and found that the crop grown in *Kharif* was superior to the *rabi* crop in terms of percentage germination and storability. They reported that percentage germination was highest in hand shelled seeds followed by seed threshed on a soft floor. Shishodia and Singh (1995) reported that seed yield of soybean decreased with delay in sowing dates, when they sowed the soybean on 22

June, 7 and 22 July and 5 August during *Kharif* season in 1992-93 at Allahabad (Uttar Pradesh). Tekrony *et al.* (1996) reported that higher levels (>30 %) of *Phomopsis longicolla* seed infection frequently occurs for the April and May sowing dates which often resulted in lower (< 85 %) seed germination for earlier maturing cultivars. Further, they concluded that high quality soybean seed can be produced on early maturing cultivars grown outside the range of adaptations, if seed infection by *P. longicolla* is controlled. Seed quality was determined in various AVRDC improved soybean lines produced in summer and autumn in various conditions. Germination test showed that the seedlots produced in summer had poorer quality than those produced in autumn. Difference between lines was evident and more pronounced in the summer than the autumn. Small seeded lines had the best seed quality even in the summer crop. There was no relationship between seed quality and seed size (Anonymous 1988).

Burchett *et al.* (1985) reported that the environmental factors prevalent during growing season frequently results in soybean seed with high incidence of seed coat etching. Paschal and Ellis (1978) studied 24 soybean collections and reported that the two weeks delay in harvest reduced the stand and field emergence by 7 and 14 % and four week delay reduced stand and field emergence by 12 and 37 %, respectively. The incidence of fungi increased from 9 % in seed examined at maturity to 21% in the seed from the 2 weeks delayed harvest and 45% in the seed from 4 weeks delayed harvest. Eweida *et al.* (1984) reported that oil content of different soybean cultivars changes according to the locations. Vaughan *et al.* (1989) reported that early maturing, maturity isolines were associated with increased infection by *Phomopsis* spp., which is related to favourable environmental conditions, such as warm temperatures and high humidity for the

spread and growth of this pathogen in the early season. Further, they reported that delayed maturity in male sterile and depodded soybeans resulted in increased infection by pathogens, probably by lengthening the period during which infection and colonization could take place. Horlings *et al.* (1991) conducted a field trial during summer and autumn seasons using lines with different seed size and seed coat characteristics. The decline in germination observed in this study was not consistently related to electrolyte leakage, *Phomopsis* infection or seed density. Performance of black seeded type with hard seed coat was similar to that of a small yellow seeded type with a rapid rate of imbibitions. In summer season, standard germination of seed from the top portion of large seeded type was near 80 % at R-7 but declined to near 50 % by R-8. Sahoo *et al.* (1991) observed that seed yield of soybean cultivar was positively correlated with cumulative mean daily temperature and mean photoperiod and negatively correlated with sunshine hours.

Nian *et al.* (1996) observed that there was significant genotype, location and genotype x environment interaction effects. Genotype x environment interactions effect were smaller than genotype and location effects. Tyler (1997) observed that seed quality of cultivars that matures early was often poor, while studying the early maturing varieties. He concluded that impermeable seed coat was not consistently effective in protecting seed quality in early maturing lines.

El-Habbal *et al.* (1991) sprayed soybean at flowering with 0, 1000 or 1500 ppm ethephon and harvested at 100, 110, or 120 DAS. Percentage seed germination after 2-12 month storage was highest in seed from crops sprayed with 1500 ppm and storage period had no consistent effects on germination percentage. Arulnandhy (1987) suggested that application of Benomyl and or Captan bi-

weekly from mid flowering to maturity significantly reduced field weathering of seeds. Emergence rate, seedling length and seedling dry weight were also significantly reduced and the half-life period of seeds stored under ambient humid tropical condition was extended to 60 days. Reddy *et al.* (1993) investigated the influence of season, genotypes and seed harvest methods on seed viability, vigour and storability of the soybean seed during two crop seasons and found that among the different genotypes included, Moneta, Bragg and KSHB 2 were better storer than Hardee and PK 471. Under Bangalore conditions of soybean seeds produced in season 1 (Aug. - Nov.) were found superior to those in season (Dec. - April).

C) Screening for resistance to mechanical damage

Mechanical injury to soybean seed has been a recurring problem in soybean producing areas. The seeds of soybean have only moderately thick seed coat. The radical or embryonic root has practically no protection except that provided by seed coat (Justice and Bass 1979). Thus soybean seeds are vulnerable to mechanical damage which affect viability of the seed. The cultivars highly resistant to mechanical damage can be developed through breeding programme by including the lines screened for resistance to mechanical damage. The difference in degree of resistance to mechanical damage of soybean lines is a genetic character. Carbonell and Krzyzanowski (1995) reported that the resistance is a genetic character that varies among soybean cultivars. The difference in resistance to mechanical damage could be ascribed to the lignin content, seed size of soybean cultivars. Lignin content of soybean seed coat plays an important role in resistance to mechanical damage (Alvarez *et al.* 1997). The seeds having high lignin content had high resistance to mechanical damage. Roberts (1972) stated that the small seed generally escape injury caused due to

mechanical damage during harvesting handling and processing, whereas large seeds are more likely to be extensively damaged. Similar statements were made by Bewley and Black (1978) who reported, that small seeds tend to escape injury during harvest and seeds that are spherical tend to suffer less damage than elongated or irregularly shaped ones. Paulsen (1978) reported that the force required to initiate seed coat rupture decreased as soybean mc increased from 8 to 17%. Maximum toughness occurred in the 11 to 14% moisture range indicating an optimum moisture range for absorbing compressive energy. Costa *et al.* (1987) tested the varietal differences for resistance to physical damage and reported that highly significant varietal differences were seen for percentage of broken seed. They further reported that the test was not capable of separating the cultivars with regards to seed resistance to mechanical damage.

Carbonell and Krzyznowsky (1995) developed the pendulum test for screening soybean genotypes for seed resistance to mechanical damage and obtained the best results with the 13 cm height impact. They separated cultivars into three categories of resistance to mechanical damage i) Resistant ii) Moderately resistant iii) Susceptible. Further, they reported that, the results confirmed the feasibility of using the pendulum test to identify genotypes with resistance to mechanical damage. Alvarez *et al.* (1997) investigated the genetics of mechanism of resistance to mechanical damage in soybean seed and concluded that higher the level of lignin content in seed coat the higher the level of resistance to mechanical damage. Couto and Alvarenga (1998) subjected the seeds of soybean cv. UFV-5, UFV-80-135 and UFV-80-354 of 10.8, 12.8 or 14.8% mc to impact from heights of 5, 10, 15, 20 and 25 cm to hilum region and found that UFV-80-135 had the largest seed and the greater resistance to mechanical damage when

the impact was to the hilum or opposite the hilum. There was no clear pattern of the effect of height of drop on damage. Shelar (2002), screened 256 lines of soybean for resistance to mechanical damage, the lines *viz.*, DS 59, DS-61, DS-108, DS-138, DS-139, DS-154, DS-94, DS-95, DS-120, DS-130 and DS-143 exhibited high resistance to mechanical damage.

D) Stacking height, Loading and unloading

Looking to the fragile nature of soybean seed coat the stacking height of soybean seed bags in godowns is also a problem. The seeds which already had received bruises, small cracks and internal cracks may result in deepening of the same. Loading, unloading and transportation had no significant effect on mechanical damage and seed quality aspects *viz.*, germination, root shoot (RS) length, vigour, moisture content, dry matter content, electrical conductivity, leaching of sugars and TZ test of soybean seeds of var. JS-335. However, there was only numerical decrease in seed quality aspects of soybean seed before loading and after transportation and unloading. It could be ascribed to less traveling distance (40 km) of the seed. However, at most care should be taken while transporting soybean seed for long distance in bulk quantity (Shelar, 2002). As pointed out by Harrington (1972), seeds are in storage from the date of their physiological maturity until they are planted. Thus, the seeds in transit are in fact in storage. As used here, the expression "in transit" includes not only the time the seed are being moved from one location to another but also the time they are awaiting in a warehouse, rail road, etc. While in transit the seeds are subjected to the same storage principles as seed in warehouse. The principal difference between warehouse storage and in transit storage are the relatively fast changing in the seed environment that can result from transportation and failure of personnel to

predict all the hazardous conditions to which seeds may be subjected.

Cabrera and Lansakara (1995) stored bags of soybean seed in stacks in warehouse under ambient conditions and reported that seed moisture content fluctuated with seasonal change in relative humidity (RH) and temperature. Greater fluctuations were observed in seeds at the top than at the middle or bottom of the stack of bags. Seed moisture content (mc) was lower in June, July and Aug. and higher during the rest of the year. Seed germination declined drastically over the period, which represents the last 12 months of a typical 19 month carryover period from 82-75 % to 29-20 %. Shelar, 2002 reported that germination of the seeds at the top of the stack was significantly higher than that of seeds from either the middle or bottom of the stack. This was attributed to the lower mc of seeds on the top of the stack during the warmer months inspite of the effects of the high temperature in that part of the stack during the summer. The mechanical damage was found to be higher to the seeds stacked at 8th layer. There was slight increase in mechanical damage in 2nd month of storage. The increased mechanical damage could be ascribed to weight of the bags in the stacks at 1 to 7 layer (Shelar, 2002). The significant effect of stacking height on different seed quality parameters of soybean seed var. JS 335 were observed during storage. The lower germination of the seed stacked at 8th layer and at 2nd month of storage than seed stacked at 1 to 7 layer could be ascribed to higher mechanical damage to the seeds stacked at 8th layer. Further, the reduction in germination might be the cause of senescence or ageing of the seed. In addition to this, the cracks of mechanically damaged seeds permit early entry of mycoflora which reduces the viability of seed. Ultimately, the RS length, vigour index and dry matter content of seedlings of seed was reduced. The EC, LS and mycoflora increased

which is negatively correlated with the viability. There was higher fluctuation in moisture content of seed stacked at 2nd layer which could be ascribed to more exposure of upper layer (2nd) to atmosphere than the 8th layer. Since the temperature was higher and relative humidity was lower during the period of storage, the moisture content had reduced in 2nd month of storage after stacking. However, there was no much difference in moisture content of seed stacked at different layers. Cabrera and Lansakara (1995) observed greater fluctuations in moisture content of seed

at the top than at the middle or bottom of the stack of bags. Germination of seeds at the top of the stack was significantly higher than that of seed from either middle or bottom of the stack.

The knowledge of the research findings on invigoration, post harvest handling of soybean seed, screening of soybean lines for resistance to mechanical damage and their use breeding programme can be useful for improving the seed quality of soybean during storage and planning of new experiment.

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