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Stepwise Hydration of Soybean Seed to Prevent Soaking Injury and Loss of Viability during Storage

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Soaking of soybean (*Glycine max* cv. Bragg) seed for a brief period followed by drying back failed to improve its germinability due to rapid imbibition and consequent damage. Stepwise hydration of the seed was, therefore, attempted to reduce membrane damage and leakage loss during hydration. Osmotic control by two-step hydration (osmotic treatment with PEG — soaking in water) followed by drying showed improvement in immediate germination, whereas three-step hydration (moisture equilibration — osmotic treatment with PEG — soaking in water) followed by drying showed remarkable effect on storability; soaking-drying treatment of humidified seeds also showed promising effect.

Key Words: Osmoconditioning, Moisture equilibration, Polyethyleneglycol, Vigour, Conductivity of seed leachate, Membrane damage

Introduction

Germination requires that seeds be hydrated. Hydration may occur as absorption from liquid water or it may, in certain instances, suffice for the seeds to be placed in a humid atmosphere, where they will gradually establish a level of hydration suitable for germination. Seed soaking, a practice often resorted to by seed growers for hastening germination and making it more uniform (Kay et al. 1977), does not always bring about the desired results. In fact, in legume seeds, (particularly in soybean), soaking directly in water is seldom beneficial (Barton 1950a, b, Orphanos & Heydecker 1968) as rapid imbibition can injure seeds (Powell & Matthews 1978, 1979). Barton (1959a, b) observed that high concentration of oxygen may cause severe soaking injury. In such cases, regulating the process of hydration *per se* by osmotica has been attempted by several workers with varied degrees of success (Khan 1977, Heeperly & Sinclair 1977, Khan et al. 1978). Soaking-drying treatment of stored seed is reported to maintain viability in several cereals, oilseeds and vegetable seeds in storage (Basu 1976). It is now becoming apparent that hydration of a dry seed preparatory to germination is after all not a simple and straightforward phenomenon and that physico-chemical

forces thus initiated in the material and structures of the seed have a role far more significant than hitherto appreciated. This paper presents several methods of preconditioning soybean seeds with a view to overcoming possible soaking injury during treatment and thus prolonging storage life and vigour.

Materials and Methods

Soybean seeds (*Glycine max* L. cv. Bragg) harvested in March, 1982 were cleaned and dried to 9.0% moisture (w.wt.). They were stored in sealed glass bottles (2.5 litres) at room temperature (av. 30±3°C) till the time of treatment.

Seed Treatment

The soaking-drying treatment was carried out according to the following methods. The technique is shown in figure 1.

A. Water soaking-drying (WS-D). Seeds were soaked in distilled water double their volume for 3 hr at 28°C, and then dried back to their original moisture content.

B. Polyethylene glycol-water soaking-drying (PEG-WS-D). Seeds were soaked in different concentrations (2–30%) of polyethylene glycol (mol. wt. ca 6000) solutions double their volume at 28°C for 2 hr followed by soaking for an additional hour in distilled water, and then dried back to their original moisture content.

C. Moisture equilibration-water soaking-drying (ME-WS-D). Seeds were subjected to moisture equilibration for 48 hr in a saturated atmosphere (100% RH) at 28°C following the method of Dey and Basu (1982). These were then treated as in A; that is, the full treatment was moisture equilibration-water soaking-drying.

D. Moisture equilibration-polyethylene glycol-water soaking-drying (ME-PEG-WS-D). Seeds were treated as in B except that before soaking in PEG and distilled water, seeds were equilibrated in a saturated atmosphere at 28°C for 48 hr, that is, the full treatment was moisture equilibration-polyethylene glycol-water soaking-drying.

Before drying, one part of each treated lot was placed for germination following the glass plate method of Punjabi and Basu (1982). The other part was dried in a current of hot air at 35 ± 1°C to its original moisture content and stored. Determination of water absorbed during different stages of soaking was made following the method of Knypl and Khan (1981). Initial moisture content of the seed and moisture level attained after equilibration in a saturated atmosphere were determined by oven-drying method.

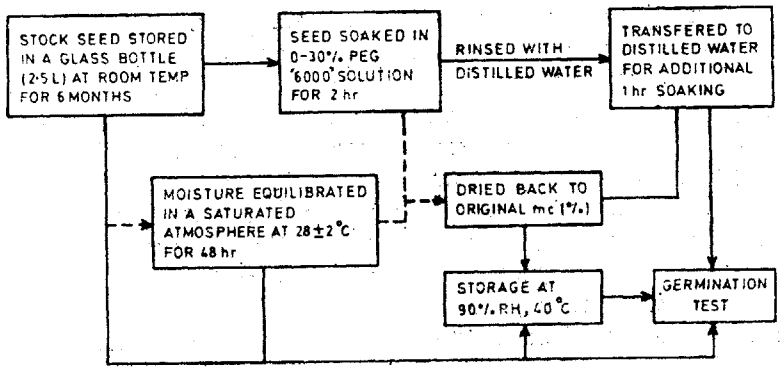

Figure 1 Methods of osmotic treatment of soybean seed

Leakage of metabolites at different steps of hydration of equilibrated and nonequilibrated seeds was tested following the method of Dey and Basu (1982).

Results

Germination and seedling growth were found to be better in moisture equilibrated seeds as compared to dry seeds directly placed for germination (figure 2). Water soaking failed to show any detectable improvement in germination over dry placement or moisture equilibration of seeds. Pretreatment of seeds with PEG '6000' (2–30%) for 2 hr followed by 1 hr soaking in distilled water improved percentage germination over pretreatment with water alone (0% PEG). However, only at concentration above 20% PEG, germination was greater than after ME alone. Polyethyleneglycol at 22.5 and 30% showed the maximum beneficial effect.

Seed vigour (seedling growth) was improved by ME alone over dry control or 3 hr water soaked control (figure 2). Treatment with PEG improved shoot length up to 22.5% PEG beyond which the effect declined. Little difference in root length was noticed between moisture equilibrated seeds and seeds treated with the higher concentrations of PEG (22.5 or 30%).

When moisture equilibrated seeds (m.c. 21.0%, w.wt.) were soaked for 3 hr in water or in different concentrations of polyethyleneglycol solutions for 2 hr followed by an additional 1 hr soaking in water, an enhancement of germination was noticed in all concentrations of PEG over only moisture equilibrated or untreated dry controls. Although the treatments were better than PEG alone, the effects were found to depend on the concentration of PEG, maximum improvement being noted at 2 and 5% with the effect declining at higher PEG concentrations. The vigour of the seedlings was better in ME followed by PEG than in ME followed by water treatment. The improvement in root length followed the pattern of germination and declined at and beyond 10% PEG; shoot

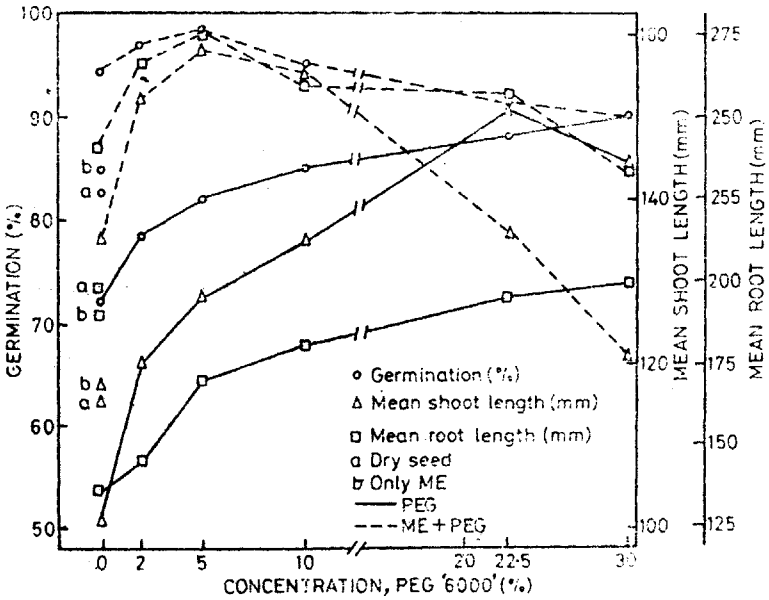

Figure 2 Effect of osmoconditioning and moisture equilibration on germination percentage and seedling vigour of soybean seeds following treatment

length showed a marked decline at 22.5 and 30% PEG.

In order to study the effect of treatments during subsequent storage the treated seeds were dried back and subjected to accelerated ageing for 6 days at 90% RH and 40°C to obtain results in a short period. The osmotically treated seeds now showed much lower germination and poor seedling growth as compared to the untreated control (figure 3). Seed soaking or moisture equilibration followed by drying was also found to be deleterious for vigour and viability under accelerated ageing. On the other hand, treatment of pre-equilibrated seeds with different concentrations of PEG proved beneficial in retaining higher germinability and better seedling growth even after drying and storage. ME followed by soaking in water (0% PEG), showed appreciable effect in the maintenance of vigour. Maximum beneficial effect was observed when pre-equilibrated seeds were treated with 2–5% PEG. Higher concentrations of PEG (22.5–30%) proved detrimental to the maintenance of viability.

Release of electrolytes by leaching during seed imbibition was highest in seeds which were not preconditioned. Such leakage was considerably reduced following equilibration or osmoconditioning of seed (table 1). Leakage was minimum at higher concentrations (22.5 and 30%) of PEG; at lower (2–5%) PEG concentrations, leakage could be reduced effectively if the seeds were equilibrated with a saturated atmosphere prior to

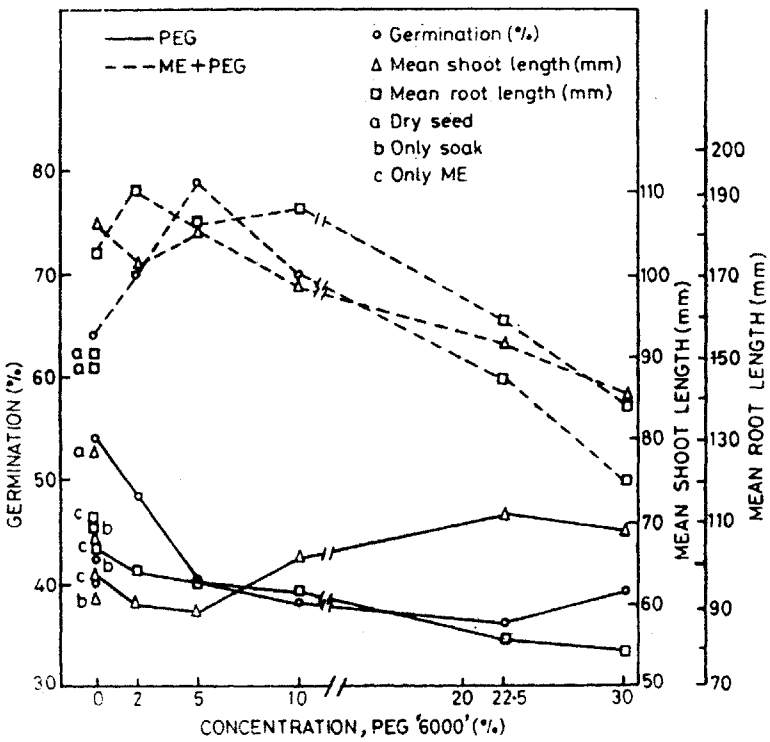

Figure 3 Effect of osmoconditioning and moisture equilibration followed by drying on viability of soybean seeds stored for 6 days at 90% RH and 40°C

Table 1 *Effect of moisture equilibration and osmoconditioning on membrane integrity during soaking. Forty seeds soaked in 20 ml of water/PEG-6000 in each of four replicates for 2 hours and transferred to water for an additional one hour. Seed equilibrated in a water-saturated atmosphere at 28°C for 48 hrs*

Treatments (Soaking)	Conductivity of seed leachate, μmhos			
	Non-equilibrated		Equilibrated	
	Initial soaking for 2 hrs	Further soaking in water for 1 hr	Initial soaking for 2 hrs	Further soaking in water for 1 hr
Water	772	124	565	200
PEG (%)				
2.0	717	143	303	209
5.0	598	109	203	200
10.0	367	112	174	208
22.5	227	146	107	210
30.0	160	146	62	230

osmoconditioning. The latter also gave the most beneficial effect on prolonging storability of seeds. It appears that maximum leakage of electrolytes from seeds occurs during the first two hours, since an additional hour of soaking in distilled water following a 2-hr treatment produced no appreciable effect.

The moisture uptake of PEG-treated seeds (both nonequilibrated and moisture-equilibrated) after 2 hr of soaking was less than the untreated water soaked seeds especially at higher concentration of PEG (table 2). Moisture equilibrated seeds had a much higher moisture content than nonequilibrated seeds without or with PEG after initial 2 hr soaking. However, after 1 hr of further soaking in distilled water the moisture content of untreated and PEG treated seeds attained almost the same level (49.5 to 51.4 in nonequilibrated; 57.1 to 60.7 in moisture equilibrated seeds). Again, the moisture equilibrated seeds treated with or without PEG, showed a much higher moisture content than nonequilibrated seeds. It may be mentioned here that the preequilibrated seeds when placed in higher concentration of PEG, absorbed less moisture; however when these were again placed in distilled water moisture uptake was quite rapid as compared to seeds which were equilibrated, then placed in lower concentration of PEG followed by soaking in distilled water, to reach the final moisture content.

Discussion

The results show that the treatments that ensured slow hydration of seeds improved seed germination and seedling growth. Moisture equilibration (ME) and polyethyleneglycol treatment either alone (PEG) or following moisture equilibration (ME-PEG), were better than water-soaking alone (WS), with ME-PEG-WS having the slowest imbibition and thereby the greatest improvement. In other words, the imbibitional injury to seed and/or seed structure during soaking directly in water is due to rapid entry of water (Powell & Matthews 1978). When seeds or their embryonic axes are placed for soaking in water, rate of imbibition is very rapid and injury is caused due to anoxia (Orphanos & Heydecker 1968), sharp decrease in enzyme activity (Duke & Kekefuda 1981) and destructive changes in ultrastructure (Powell & Matthews 1979). In fact, Woodstock and Taylorson (1981) have shown that just one-hour soaking of soybean seeds can markedly inhibit germination and seedling growth. Our results also confirm these observations.

Osmotic treatment in the PEG reduces imbibitional or chilling injury in legume seeds (Tulley et al. 1981). In low vigour seeds of soybean, soaking of seeds in 30% PEG could effectively avoid soaking

Table 2 *Moisture changes in (dry and moisture equilibrated) seed during osmoconditioning. Dry untreated seed did not receive any humidification; moisture equilibration done in a water-saturated atmosphere for 48 hours. Data presented in parentheses show the increase in moisture content (% w.wt) over untreated control. Other details same as in table 1*

Treatments (Hydration)	Non-equilibrated, % MC, w. wt.			Moisture-equilibrated, % MC, w.wt.		
	Initial	After 2 hour soaking	Further 1 hour soaking	Initial	After 2 hour soaking	Further 1 hour soaking
Untreated	9.1			—		
Moisture equilibrated	—			21.1		
Water soaked		44.3 (35.2)	51.4 (42.3)		54.0 (45.0)	57.4 (48.3)
Soaked in PEG (%)						
2.0		42.1 (33.0)	50.9 (41.8)		55.0 (46.9)	60.0 (50.9)
5.0		41.1 (32.0)	51.0 (41.9)		56.1 (47.0)	60.7 (51.6)
10.0		38.5 (29.5)	49.9 (40.8)		53.3 (44.2)	59.1 (50.0)
22.5		34.4 (25.3)	49.5 (40.4)		47.2 (38.1)	57.4 (48.3)
30.0		31.2 (22.1)	50.4 (41.3)		46.3 (37.2)	57.1 (48.1)

injury and improve germinability and vigour of treated seeds (Woodstock & Taylorson 1981). Bramlage et al. (1977) have reported that humidification of soybean seeds before imbibition reduced leakage and chilling injury during soaking. Saha and Basu (1982, 1984) have shown that soaking of PEG-treated seeds would improve immediate germinability as well as storability in soybean. In the present investigation it has been found that the treatment ME-PEG-WS is most promising in improving immediate germinability as well as storability of a seed lot. Beneficial effect of moisture equilibration in reducing membrane leakage was reported earlier by Rudrapal and Basu (1979) and Dey and Basu (1982) who suggested a control of free radical-mechanism induced membrane damage in this treatment. Basu and Dasgupta (1978) have shown that the moisture content of seeds attained during moisture equilibration alone (20–25%) was sufficient to prevent the damage due to free radical reactions in wheat seeds. But in soybean seeds only moisture equilibration-drying treatment failed to maintain viability under storage.

The higher moisture content of seed reached during the ME-PEG-WS treatment, unaccompanied by any severe leakage loss, may be necessary for counteracting the free radical pathology or lipid autooxidation damage during storage. Such treatments may also initiate or improve repair mechanism and prevent deterioration (Villiers & Edgcumbe 1975, Hegarty 1978).

Thus moisture equilibration followed by osmoconditioning which showed smooth

entry of water into the seed unaccompanied by soaking injury to membrane, possibly without affecting membrane reorganisation, considerably improved immediate germinability and retained viability of the soybean seed for a longer period.

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