



Review

Seed Priming: Triumphs and Tribulations

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Priming has a long and chequered history dating back to time of Greeks. Seed priming has shown promise in enabling seeds to overcome biotic and abiotic stresses. Simple priming techniques using salt solutions gave way to elaborate and sophisticated media that warrant close monitoring. Biopriming of late has been practiced for making priming eco-friendly. Elucidation of molecular basis has shown the cause for invigoration but confirmation of DNA's role is yet to be done. Activation of preformed enzymes in providing vigor seems putative. Development of tests to determine primed seed quality is urgently warranted. Addressed in the paper are the ways and means for assessing the quality of primed seeds. Methods to store them for long periods are necessary conserve the benefits and market primed seeds to promote their use over time and space.

Key words: Seed priming, biopriming, priming techniques, advantages, disadvantages.

Consequent to germination, good crop establishment is a major constraint to crop production in the semi-arid tropics (Matarira *et al.*, 2004). Seeds and seedlings often experience adverse physical conditions in the seedbed viz., mechanical resistance, high temperatures, high solute concentration at seed-water interface leading to peak osmotic potential and rapid soil drying and crusting (Parera and Cantliffe, 1994). Often slow, asynchronous, unreliable germination, delayed emergence and insufficient stand establishment are major problems in low-precipitation areas.

Seed priming is an age old practice, practiced eons ago by Greeks. The word was coined by Heydecker in 1973 for the soaking drying seed treatments. Theophrastus (372-287 BC) had recommended presoaking of cucumber seeds in milk or water to make them germinate earlier and vigorously (Michael Evanari, 1984). Later, Heydecker (1973) successfully used seed priming to improve germination and emergence under stressful conditions. This technique is a treatment applied prior to sowing in a specific environment wherein seeds are partially hydrated to a point of germination process initiation sans visible symptom of radicle emergence (Bradford, 1986; Dell Aquila and Tritto, 1991; Kaur, 2002; Giri and Schilinger, 2003).

During seed priming the amount of water absorption is controlled so as to allow necessary metabolic activities for germination to take place but prevent radicle emergence by limiting the seed water content. Different physiological activities occur within the seed at different moisture levels.

Primed seeds usually exhibit an increased germination rate, greater germination uniformity, and, at times, greater total germination percent (Basra *et al.*, 2005), improved germination under sub-optimal conditions (Lin and Sung, 2001). Increased germination rate and uniformity have been attributed to metabolic repair during imbibition (Bray *et al.*, 1989), buildup of germination-enhancing metabolites (Basra *et al.*, 2005), osmotic adjustment (Bradford, 1986), and, for seeds that are not re dried after treatment, a simple reduction in imbibition lag time (Bradford, 1986).

Harris (2006) a veteran in promoting priming concept in least developed countries, reported that large numbers of farmers have tested seed priming for themselves and found improved yield in 204 (farmers) (40% per cent) with upland rice in Cameroon, 257 (70%) in Ghana, 274 (33%) in Sierra Leone, 145 (25%) in Gambia, 40 (113%) in Nigeria and 180 (10%) in Thailand. In case of chickpea, more than 300 (40%) farmers' trials have been implemented in Bangladesh, Nepal and Pakistan. In wheat, 275 farmers in India, Pakistan and Nepal have tested seed priming (5-35%). Maize trials by 72 (29%) farmers in Pakistan and Zimbabwe were also very successful. The farmers reported that seed priming was found to be effective in semi-arid (chickpea, mungbean, sorghum, finger millet, maize), high-potential (wheat, maize) and forest agriculture interface (upland rice) production systems. The concept has been extended through participatory approaches to enable farmers to test seed priming in Bangladesh, Cameroon, Gambia, Ghana, India, Nepal, Nigeria, Pakistan, Sierra Leone, Thailand and Zimbabwe.

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Priming can also be effectively practiced to have synchronised flowering in hybrid seed production plots. In sunflower hybrid TCSH 1 a gap of about a week in flowering between the seed and pollen parent was bridged by a simple seed hydration treatment with gibberellic acid (50 ppm) and a spray of urea (1%) at button formation thrice, on alternate days. The results suggested a perfect synchronization of flowering not only in terms of first flowering in both A and R line, but also a good match in frequency of plants flowered in both A and R lines on a given day during flowering. (Chakrabarthy, 2008)

Alleviation of Thermo-dormancy

Priming also releases seed dormancy in some crops. In thermosensitive varieties of lettuce, germination is reduced or completely inhibited at high temperatures such as 35°C. But lettuce seeds primed for 20 h in 1% potassium phosphate or distilled water had up to 86% germination (Daniel, 1984). He opined that Seed priming appeared to lead to the irreversible initiation of cell elongation, thus overcoming thermodormancy. Durman (2006) also recommended seed priming with PEG 6000 or potassium phosphate (1%) for lettuce under field stress to overcome thermo dormancy.

Priming techniques

Priming is done by either uncontrolled hydration (hydro priming) or controlled hydration (osmo, matrix) (Taylor *et al.*, 1998). As water is the cheapest and abundant resource, seed tissues with their affinity to water, hydrate and dehydrate easily and respond vigorously upon priming, unless they are unviable, dormant with provision of optimal conditions (Subedi and Ma, 2005). The Physiology of seed priming is depicted in Fig. 1

Current controlled seed priming methods include osmotic priming (soaking in an osmotic such as polyethylene glycol (PEG) or salts (Kaur *et al.*, 2002a), solid matrix priming (seeds absorbing water from a water-absorbing carrier such as peat or vermiculite (Taylor *et al.*, 1988)). Off late biopriming using microbial infusion has picked-up.

1. Osmotic priming or Osmo priming:

Osmopriming (osmoconditioning) is the standard priming technique. Seeds are incubated in well aerated solutions with a low water potential, and later washed and dried. The low water potential of the solutions can be achieved by adding osmotica like mannitol, polyethylene glycol (PEG) or salts like potassium chloride.

Heydecker *et al.* (1973) defined osmotic seed priming as a pre-sowing treatment in an osmotic solution that allows seeds to imbibe water to proceed to the first stage of germination but prevent radicle protrusion through the seed coat. Different osmotica can be used in seed priming and these, according to Taylor *et al.* (1998), have got different characteristics and levels of efficacy. Some of the osmotica that can be used include potassium nitrate, potassium dihydrogen ortho-phosphate, Dipotassium hydrogen ortho-phosphate, calcium chloride, zinc sulphate, borax, magnesium chloride, manganese sulphate, sodium chloride, sodium sulphate, organic compounds *viz.*, agrosan, cycocel, citric, furamic, succinic, malic acids, purines, pyrimidines, caffeine, uracil, xanthine and uridine diphosphate (De Chandra, 1999).

Effect of priming with chemical osmotocants vary with crop and the chemical used. Richard foti *et al.*

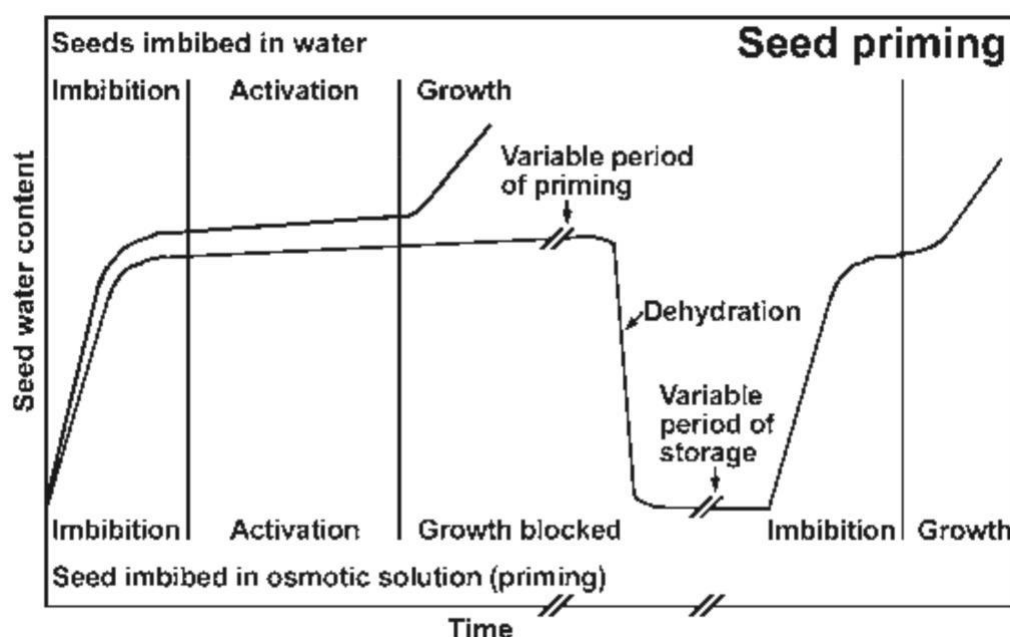


Fig. 1. Physiological basis of seed priming.

(2008) reported that osmotic priming with copper sulphate at 0.1% was the most suitable seed priming osmotica for maize. They observed increased seed emergence by 43% and 29%, due to priming in copper sulphate and zinc sulphate respectively while priming in sodium sulphate did not significantly increase emergence. Hossein Sadeghi (2011) observed significant results on soybean seed germination percentage, mean germination time, germination index and the time to get 50% germination, seed vigor and electrical conductivity of seeds due to different osmotic potential and priming duration. Osmotic potential of -1.2 MPa and 12h priming had positive results.

Lee *et al.*, (1998) concluded that priming in a - 0.6 MPa PEG solution for 4 days as optimum for rice seed priming. Both osmopriming (-1.5MPa PEG) and hydropriming for (12-36hr) improved mean emergence time under sub optimal temperature (15°C) (Moradi and Younesi, 2009). The same authors while investigating seed priming under excess moisture conditions suggested that the same treatment at 25°C produced better results. Ruan *et al.*, (2002) observed improved seedling vigor index and seedling stand establishment in flooded soil when seeds were treated with CaCl₂ and CaCl₂

+ NaCl. Seeds osmoprimed with PEG and KNO₃ solutions for 12h had improved the germination and vigour of wheat seeds (Hojat salehzade *et al.*, 2009).

Disadvantages of osmotic priming

Priming seeds in PEG requires large quantities of PEG, which increases the expense (Rowse, 1996); large quantities of PEG must be handled and disposed. Further aeration of hydration solution is must to get an adequate effect. PEG priming provides beneficial conditions for bacterial growth due to poor aeration (Parera and Cantliffe, 1994). Crops seeds seem to be chemical specific; hence research has to be done for choosing the correct chemical and its optimum dose for a crop. Managing huge quantities of wet primed seed becomes difficult especially under hot tropical climate In temperate climate maintaining the priming temperature is crucial.

2. Halo priming

Halo priming involves the use of salts of chlorides, sulphates, nitrates etc. The salts used to control water potential may cause toxicity and/or germination inhibition in rice (Basra *et al.*, 2003, 2005). In both studies, -1.1 MPa KNO₃ adversely affected the germinating seeds and seedlings. In amaranthus, seed priming with CaSO₄ + NaCl increased the salt tolerance at seedling and early vegetative stage by promoting K and Ca accumulation besides inducing osmoregulation. A brief list of seed priming research undertaken in TNAU is provided in Table 1.

Table 1. List of crops, priming techniques used and their effects

S. No	Crop	Chemicals Used For Seed Priming and their concentration	Effect on crop	Authors
1	Paddy (<i>Oryza sativa</i> L.)	KNO ₃ 10 ⁻⁴	Increased seed quality parameters and resultant seeds quality	Geetha, (1992)
		Na ₂ PO ₄ 10 ⁻⁴ Na ₂ H ₂ PO ₄ 10 ⁻⁵		
		KCl, CaCl ₂ Leaf extracts of <i>Prosopis</i> , Pungam and <i>Acacia</i> (1%)	Increased seed quality parameters	Nagaraj, (1996)
		-1.25 MPa of CaCl ₂ , NaCl KNO ₃ and KCl	Increased the field emergence, seedling fresh and dry weight	Basra <i>et al.</i> , (2004)
		PEG, Mannitol, KNO ₃ , KCl, K ₃ PO ₄ , KH ₂ PO ₄ , MgSO ₄ , CaCl ₂ and NaCl		Farooq <i>et al.</i> , (2005)
Hybrid rice ADTRH 1		-1.25 MPa of CaCl ₂ , NaCl, KNO ₃ , KCl	Increased seed quality parameters	Farooq <i>et al.</i> , (2006)
		Organic priming with Panchakavya Coconut water, Vermiwash	Increased germination	Sundaralingam, (2005)
2	Wheat (<i>Triticum aestivum</i> L.)	PEG 8000 and KNO ₃	Germination parameters	Salehzade <i>et al.</i> , 2009
3	Barley (<i>Hordeum vulgare</i> L.)	Water soaking for 12 – 16h	Priming was also more advantageous on saline-sodic than on saline soils	Rashid <i>et al.</i> , 2006
4	Maize (<i>Zea mays</i> L.)	Urea -1.2 MPa PEG -1.2 MPa	Increased seed quality parameters	Pegah <i>et al.</i> , (2008)
		GA ₃ 100ppm, IAA 100ppm ASA 50ppm, CaCl ₂ 50mM	Increased field emergence, seedling fresh and dry weight	Afzal <i>et al.</i> , (2008)
		Holy basil 15 %	vigour and viability	Vanitha, (2005)

Table 1 continued

S. No	Crop	Chemicals Used For Seed Priming and their concentration	Effect on crop	Authors
5	Sorghum (<i>Sorghum bicolor</i> (L.) Moench)	KH ₂ PO ₄ 2% DAP 2% Micronutrient mixture	Increased seed quality parameters	Selvaraju, (1992)
6	Green gram (<i>Vigna radiata</i> L. Wilczek)	Succinic acid, CoNO ₃ , Ascorbic acid, NaMoO ₄ , K ₂ SO ₄ , MnSO ₄ , ZnSO ₄ , KCl and IBA	Increased yield attributes	MuBark Basha (1982)
7	Black gram (<i>Vigna mungo</i> (L.) Hepper)	Succinic acid, CoNO ₃ , Ascorbic acid, ZnSO ₄ , KCl, IBA and K ₂ SO ₄	Increased the field emergence, stand, early flowering and enhanced germi nability of resultant seeds	Vijaya Kumar, (1982)
		KCl 1%,KH ₂ PO ₄ 1% CaCl ₂ 1%, NaCl 1% Ca Cl ₂ +NaCl+ KH ₂ PO ₄ (1:1:1), FeSo ₄ 1% GA ₃ 100ppm, KNO ₃ 0.5% Kinetin 100 ppm, IAA 100ppm IBA 100ppm	Increased seed quality parameters	Srimathi and Sujatha (2006)
		0.5 % KNO ₃ +2% Prosopis leaf extract, 0.5 % KNO ₃ +2% moringa leaf extract	Improved seed quality and shelf life of seeds	Surulirajan (2007)
8	Cowpea (<i>Vigna unguiculata</i> (L.) Hepper)	KH ₂ PO ₄ 10 ⁻⁴ , KCl 10 ⁻⁴ , DAP 1%	Increased yield attributes and shelf life of seeds	Sujatha (1994)
		Ocimum 10% Chicory 20% Calotropis 15%	Increased seed quality parameters , growth and yield attributes	Sujatha and Ramamoorthy (2008)
9	Red gram (<i>Cajanus cajan</i> L. Millsp.)	Succinic acid, CoNO ₃ , NaMoO ₄ , Ascorbic acid, K ₂ SO ₄ , MnSO ₄ , ZnSO ₄ , KCl and IBA	Improved the seed quality and yield attributes	Rajendran (1982)
10	Soybean (<i>Glycine max</i> (L) Merr)	NaCl 0.5% , NaNO ₃ 0.05%, KNO ₃ 1%, Thiourea 0.025%, DAP 0.025%	Increased growth and yield attributes	Sathiyamoorthy and Vivekanandan (1988)
11	Cotton (<i>Gossypium hirsutum</i> L.)	Ca(OH) ₂ (2/4%), KCl (1%)	Increased seed quality parameters ,field emergence and vigour of seeds	Senthilkumar (1993).
12	Sunflower (<i>Helianthus annus</i>)	0.5% , 1% and 2% ZnSO ₄ 0.5% , 1% and 2% MnSO ₄ 0.5%, 1% and 2% Sodium Molybdate KNO ₃ 0.5%, NaCl 0.1 %	Increased growth and yield attributes and oil content	Muthuvel <i>et al.</i> , (1983)
		Holy basil 15%, chicory 20%	Early emergence and increased yield attributes	Mubshar Hussain <i>et al.</i> , (2006)
		Holy basil 15%, chicory 20%	Vigour and viability	Vanitha (2005)
13	Sesamum (<i>Seasamum indicum</i>)	GA ₃ 100ppm Tamarind leaf extract 2% MnSO ₄ 0.5 %	Increased seed quality parameters	Suma (2005)
14	Mustard and Radish	Hydropriming Sand matric priming Osmopriming ,-1 MPa PEG Halopriming, KNO ₃ 3%	Increased germinability, vigour and storability	Nethaji, (2006)
15	Tomato (<i>Lycopersicum esculentum</i>)	Sodium phosphate (dibasic)	Increased the seed quality parameters and controlled the microbial activity and extending the shelf life of seeds	Sree Rama Murthy, (1984)
16	Bhendi (<i>Abelmoschus esculentus</i> L.)	CaOCl ₂ 2%	Increased seed quality parameters	Sambandhamani, (1988)

Table 1 continued

S. No	Crop	Chemicals Used For Seed Priming and their concentration	Effect on crop	Authors
17	Bhendi and Beetroot	Hydropriming Sand matrix priming Osmopriming, -1 MPa PEG Halopriming, KNO ₃ 3%	Increased germinability, vigour and storability	Nirmala (2006)
18	Bitter gourd (<i>Momordica charantia</i>)	Bavistin, Boric acid, CaOCl ₂ , KNO ₃ Mixtalol. 1% NaH ₂ PO ₄ (1.5%) KH ₂ PO ₄ (1.5%), Succinic acid 1.5%, Thiourea (0.5 %), NAA (0.01 %) Cytoszyme (0.01%)	Increased seed quality parameters	Renuga devi and Jacqueline (1994)
19	Carrot (<i>Daucus carota</i> L.)	FeSO ₄ (0.2 %) Na ₃ BO ₃ , ZnSO ₄ , GA ₃ , CuSO ₄	Increased germination and seedling growth	Ramesh 1996)
20	Onion (<i>Allium cepa</i>) and Carrot	Hydropriming, Sand matrix priming Osmopriming - 0.25 MPa PEG Halopriming 3% KNO ₃ and NaCl	Increased seed germinability vigour and storability	Selvarani (2005)
21	Cashew (<i>Anacardium occidentale</i> L.)	GA ₃ 200 & 400ppm Thiourea 5000 & 10000ppm KH ₂ PO ₄ 2500 & 5000 ppm Kinetin 20 & 40ppm NaCl- 10%, NaH ₂ PO ₄ 1000ppm	Increased the storability up to 13 months	Mini and Jose Mathew (2008)
22	Jatropha (<i>Jatropha curcas</i> Linn)	FeSO ₄ , ZnSO ₄ MnSO ₄ , CaCl ₂ , KCl	Increased germination and higher seedling survival	Kathiravan (2004)

3. Hydropriming (drum priming)

It is achieved by continuous or successive addition of a limited amount of water to the seeds. 'On-farm steeping' is the cheap and useful technique that is practiced by incubating seeds (cereals, legumes) for a limited time in warm water. A drum is used for this purpose and the water can also be applied as humid air or as water vapor hence is called as drum priming (Rowse, 1996). Chakrabarty (2008) suggested seed hydration treatment with Gibberellic acid (50 ppm) and a spray of urea (1%) at button formation thrice, on alternate days for better synchronization of flowering of parental lines of sunflower hybrid TCSH-1.

In contrast to PEG or sand priming, soaking seed in aerated water before sowing is a simple, low cost, low-risk approach; however, there is some risk of imbibition damage which results in anaerobic respiration and pathogen proliferation (Wang *et al.* 2004). Pulses are especially vulnerable to imbibition damage.

The best germination and quality of seedlings were obtained in bell pepper through pre-sowing seed priming treatments of *Melia azedarach* leaf extract 10 % followed by *Eucalyptus* leaf extract 10%, garlic clove extract 5%, cow urine 5% and cow dung extract 5% at seed soaking duration of 24 hours (Mehta *et al.*, 2010).

4. Matrix priming (matri-conditioning):

It is the incubation of seeds in a solid, insoluble matrix (vermiculite, diatomaceous earth, cross-linked highly water-absorbent polymers) with a limited amount of water. This method confers a slow imbibition. Zhang *et al.* (2007) observed significantly

weight in maize cultivars compared to unprimed control. The Waxy maize seeds were mixed with sand containing 4% (v/w) water, sealed in plastic box, and then primed at 15°C for 48 h. They also stated that sand priming had increased the content of soluble sugar and the activities of peroxidase (POD) and catalase (CAT). At the same time, the malondialdehyde (MDA) and proline (Pro) accumulation decreased under a high-salt stress condition. Sand matrix priming using 5% moist sand is recommended for soybean.

5. Biopriming

Bio-priming is a process of biological seed treatment that refers to combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organism to protect seed. It is an ecological approach using either bacteria or selected fungal antagonists against the soil and seed-borne pathogens. Biological seed treatments may provide an alternative to chemical control of crop diseases.

Bio-priming involves coating / soaking seed in slurry of bacterial biocontrol agents such as *Pseudomonas aureofaciens* Kluver AB254 and hydrating for 20 h under warm conditions (23°C) in moist vermiculite or on moist germination blotters in a self-sealing plastic bag. The seeds are removed before radicle emergence (Callan *et al.*, 1990) or presoaking the seeds in water for 12 hours and then mixed with formulated product of bioagent (*Trichoderma harzianum* and/or *Pseudomonas fluorescens*) and heaped under high humidity for about 48h at 25-32°C. List of biocontrol agents and

enhanced shoot height, seedling fresh and dry

their promotory effects is presented in Table 2.

Table 2. Benefits of biopriming in crops using biocontrol agents

Crop	Biocontrol agents	Disease control	Reference
Sunflower	osmopriming with NaCl. & Biopriming with <i>Pseudomonas fluorescens</i>	Improved germination parameters	Moeinzadeh <i>et al.</i> , (2010)
	<i>Pseudomonas fluorescens</i> (0.8%) in jelly	Alternaria blight	Rao <i>et al.</i> , (2009)
Maize	<i>Trichoderma harzianum</i>	<i>Fusarium verticillioides</i> and <i>fumonisin</i> s	Chandra Nayaka <i>et al.</i> , (2010)
Radish	Rhizobacteria Strains: <i>Agrobacterium rubi</i> strain A 16, <i>Burkholderia gladii</i> strain BA 7, <i>Pseudomonas putida</i> strain BA 8, <i>Bacillus subtilis</i> strain BA 142, and <i>Bacillus megaterium</i> strain M 3	Improved seed germination under saline conditions	Haluk Çalır Kaymak <i>et al.</i> , (2009)
Carrot	Antagonist, <i>Clonostachys rosea</i>	<i>Alternaria dauci</i> and <i>A. radicina</i>	Jensen <i>et al.</i> , (2004)
Sweet corn	<i>Pseudomonas aureofaciens</i> strain AB254	<i>Pythium ultimum</i>	Mark Bennett, (1996)
Pearl millet	<i>Pseudomonas fluorescens</i>	Downy mildew	Raj Niranjana <i>et al.</i> , (2004)

Hardening

Unlike priming, hardening is uncontrolled hydration using water involving repeated cycles of wetting and drying without radicle protrusion. Hardening is the best treatment for rainfed sowing. The hydration-dehydration cycle may be repeated several times (Lee and Kim, 2000). The hardening treatment for 24 h proved to be better for vigor enhancement (Basra *et al.*, 2005) than osmopriming (-1.1 MPa KNO₃) for 24 and 48 h and traditional soaking (overnight soaking followed by saturated gunny bags up to radicle appearance). Basra *et al.* (2003) also evaluated the effects of seed hardening for 24 and 18 h and reported that this resulted in better invigoration in hardened seeds of fine rice compared with osmoconditioning and the control. Greater σ -amylase activity and higher sugar content were also reported in the hardened seeds than in the control.

Farooq *et al.* (2005) introduced a new technique for rice seed invigoration, integrating both seed hardening and osmo-conditioning. Seeds of coarse and fine rice were hardened in various salt solutions rather than in tap or distilled water. Osmohardening in CaCl₂ (with an osmotic potential of -1.5 MPa) solution was found to be better than with other salts and simple hardening (Farooq *et al.*, 2005).

Humidification is a presowing hydration treatment in which seeds are equilibrated under conditions of high humidity (Lee *et al.*, 1998b). Humidification of normal rice seeds with a high germination rate did not increase the germination rate, but did accelerate the germination rate of aged

seeds, especially those under unfavorable soil conditions and suboptimal temperatures (Lee *et al.*, 1998a,c). Lee *et al.*, (1998b) investigated the effects of humidification on normal and aged rice seeds. Relative humidity (RH) and humidification duration were found not to affect germination rate, but they reduced the time to 50% germination of normal seeds. Humidification at 60% RH did not affect germination rate or time to 50% germination of aged seeds, but that at 80% RH reduced germination percentage and increased the time to 50% germination (Fig. 2)

Physiological and molecular basis of priming

1. Stages of water uptake during germination where priming is relevant

The pattern of water uptake during priming is similar to that during germination but the rate of uptake is slower and controlled to prevent radicle emergence.

2. Changes in protein profile

Proteomic analysis in Arabidopsis revealed that new proteins are involved either in the imbibition process of the seeds (such as an actin isoform or a WD-40 repeat protein) or in the seed dehydration process (e.g. cytosolic glyceraldehyde-3-phosphate dehydrogenase) which helps to characterize seed vigor of commercial seed lots and to develop and monitor priming treatments (Gallardo *et al.*, 2001).

A considerable increase in the polypeptide bands of soluble protein at 20.1 kDa and 26.7 kDa regions and also considerable increase in the heat

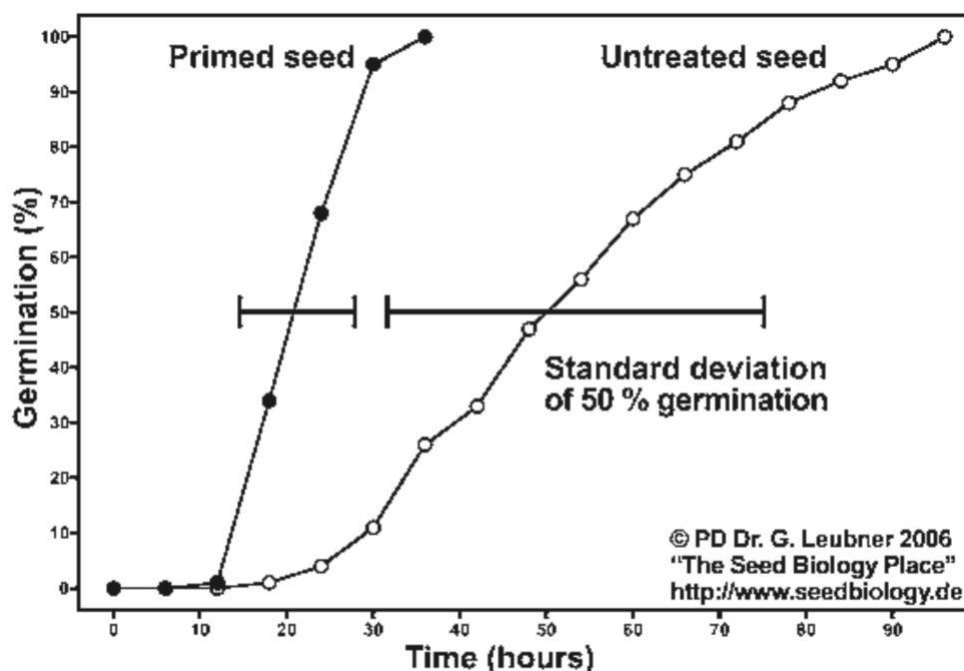


Fig. 2. Germination rate improvement in primed seeds

stable protein at 66-kDa region were observed in the primed seeds. In addition to the soluble protein at 20.1-kDa and 26.7-kDa regions and heat stable protein at 66-kDa region, the total protein and soluble protein at 50.6-kDa region was also found abundantly in protein extracts of UMI 61 and COH(M) 5 maize seeds (Sathish, 2009).

Lee and Kim (2000) investigated the effects of osmoconditioning on germination of normal and naturally aged seeds by analyzing total sugar content and α -amylase activity. They observed higher total sugar content and α -amylase activity in normal seeds than the aged seeds. Aged seeds that underwent osmoconditioning and hardening increased their total sugar and α -amylase activity. The latter was positively correlated with total sugar and germination rate. Basra *et al.* (2005) had the same results when they evaluated the effects of osmoconditioning (-1.1 MPa KNO_3 for 24 and 48 h), traditional soaking and toxic effects of KNO_3 osmoconditioning on fine rice. Increased α -amylase activity and sugar content were reported in the treated seeds compared with the control.

Reduction in cell phytate content and stored minerals viz., boron, copper, magnesium, manganese, iron and zinc with concomitant increase in potassium was also found with 1% KH_2PO_4 (6h) osmoconditioned seeds of maize hybrid COH(M) 5 (Sathish, 2009)

Anuradha Varier *et al.*, (2010) opined that some proteins were synthesized only during priming and not during germination eg. the degradation products of certain storage proteins (such as globulins and cruciferin) are detected only during priming and not

when imbibed in water. A possible explanation is that the slight water stress situation created during priming (particularly osmoconditioning) can induce the breakdown of these proteins thus initiating the process of reserve protein mobilization earlier than in the unprimed seed.

3. Enzyme activation in relation to priming

Activities of several enzymes associated with the germination process have been observed to change in response to seed priming. These include increases in activities of α -amylase in rice (Basra *et al.*, 2005, Farooq *et al.* 2006b), acid phosphatase and esterase in lettuce (Khan *et al.*, 1978), and antioxidant enzymes in lucerne (Zhang *et al.*, 2007). A rapid resumption of DNA synthesis and initiation of cell division was observed in wheat soon after hydration (Dell'Aquila and Taranto, 1986) while repair of DNA and other cellular components (e.g. membranes), which may be damaged during seed maturation, dehydration and storage, has been suggested to take place during seed priming. It has also been suggested that the onset or completion of DNA repair may be a major contributing factor to the improvement in germination after osmoconditioning (Burgass and Powell, 1984).

Primed seeds had less lipid peroxidation and higher superoxide dismutase (SOD) and catalase (CAT) activities than non-primed rice seeds. Amylase activities and starch breakdown were also hastened in primed seeds (Evangelina *et al.*, 2011). He also opined that survival after flooding was positively correlated with amylase activity but negatively correlated with the extent of lipid peroxidation. Protein sugar and RNA were found to increase in PEG

treated seeds of cauliflower (Fujikura and Karsen, 1992). Enzyme activities of catalase, peroxidase, amylase and invertase increased in PEG treated seeds (Singh *et al.*, 1985).

Advancement of radicle meristem cells into the S and G₂ phases of the cell cycle, increasing the percentage of nuclei having a 4 C DNA content, has been reported to occur during priming. Sunitha Gurusinge (1998) reported that replicative DNA synthesis in radicle meristem nuclei often occurred during tomato seed priming, but an increase in the percentage of 4 C nuclei was not seems to be essential for germination advancement.

4. Priming - molecular basis

Several researchers have contributed in elucidating the molecular basis for DNA priming. Earlier doubts of Coolbear and Grierson (1979) about the unchanged DNA content of tomato were put to rest by latter day researchers. Increase in DNA synthesis (Bray *et al.*, 1989 (Leek); Dell'Aquila and Taranto, 1986 (wheat); Coolbear *et al.*, 1990 and Yongging *et al.*, 1996 (tomato cv. Moneymaker); Lanteri *et al.*, 1994 (pepper); Asraf and Bray, 1993 (Leek); Gurusinge *et al.*, 1999 (tomato)) and DNA repair (Fu *et al.*, 1988) have been reported.

Coolbear *et al.* (1990) working with primed tomato seed cv. Moneymaker reported reduced rate of nucleic acid accumulation and reduced RNA polymerase activity per unit DNA, implying that rRNA synthesis within these seeds was under some measure of stringent control.

Clarke and James (1991) reported that there was no net increase in RNA and DNA content of leek seeds during osmopriming. However, the ratio of RNA and DNA correlated with the germination performance of four lots of seeds studied, and proposed that such a relationship is indicative of the efficiency of a priming treatment, and may be useful in comparisons of naturally varying seed lots. Priming also prepares the cell for division by enhancing the synthesis of β -tubulin which is a component of microtubules. These effects of priming are retained even after drying the primed seed. The exact mechanism by which priming regulates the cell cycle needs to be investigated (Anuradha Varier *et al.*, 2010).

Chiu *et al.* (1995) reported that improvement in germination by priming might be due to enhanced repair of membrane, which was disrupted during maturation drying. This was indirectly supported by the reduced leakage of electrolytes from primed seeds, since electrolyte leakage is in part a result of damage cell membranes. However electrolytes may be leak out during priming, resulting in lower levels of electrolytes in primed seeds than in control. A time course basis of molecular activity was provided by Anuradha Varier *et al.* (2010)

Sathish (2009) observed no significant difference with the DNA content up to 6 h of germination due to priming. However at 12 h of germination, two fold increase in the DNA content of seeds primed with KH₂PO₄ 1% for 6 h was observed in maize genotypes. Subsequently, he observed lesser rate of increase in DNA content at 24 and 48 h of germination compared with the rate of increase at 12 h of germination.

Factors affecting priming

Priming must be affordable and practicable by farmers for its easy spread and adaptability. Improvement in priming is affected by many factors such as plant species, water potential, priming factor, priming duration, temperature, vigor and primed seed storage condition (Khan 1992; Parera and Cantliffe, 1994; McDonald, 1999; Ruan and Xue, 2002 and Mubshar *et al.*, 2006). The following factors will determine the success of priming in any crop (Anuradha Varier *et al.*, 2010).

Aeration: Provision sufficient air by pumping air during priming is important as it accelerates cell rejuvenation.

Light: Celery and lettuce responded to priming done with light compared to other crops

Time: Duration of priming depends on seed, temperature, osmotica and osmotic potential of solutions

Temperature: Temperature must be optimum to minimize radicle protrusion.

Method: Choosing the right type of osmotica which must be non-toxic, chemically pure, amenable for maintaining correct osmotic potential, free from pathogen invasion, economical and easily available.

Priming of stored seeds - low vigour seeds

In general, priming improves the longevity of low vigour seeds, but reduces that of high vigour seeds. The high vigour seed is at a more advanced physiological stage after priming nearly at stage III, and thus more prone to deterioration. When a low vigour seed is primed, it requires more time to repair the metabolic lesions incurred by the seed before any advancement in germination can occur, thus preventing further deterioration.

Priming is also responsible to repair the age related cellular and subcellular damage of low vigor seeds that may accumulate during seed development (Bray, 1995). Priming of seed promotes germination by repair of the damaged proteins, RNA and DNA (Koehler *et al.*, 1997).

Bailly *et al.* (1998) reported that priming of aged sunflower seeds in PEG progressively restored the initial germinability and resulted in marked decrease in the level of MDA and conjugated dienes, indicating a fall in lipid peroxidation processes. Kathiresan *et*

al. (1984) found that priming of aged sunflower seeds with CaCl_2 , KH_2PO_4 , NaCl , ZnSO_4 , ascorbic acid and succinic acid improved emergence and seedling growth of seedlings.

Kausar *et al.* (2009) observed effectiveness of priming with $\text{K}_2\text{H}_2\text{PO}_4$ in all germination related parameters in low vigour sunflower seeds. Priming of normal/low-vigor seeds improved the vigor of seedling in term of radicle length, plumule length and their root/shoot fresh weight. After cold stress low-vigor-primed and normal-primed exhibited significant change in all emergence parameters except E50 of normal and low-vigor seeds. They also observed some proteins which were completely lost as a result of AA, which showed reappearance after priming. The proteins with 26, 48, 49, 69, 90, 118, 121, 150 and 199 kDa were affected by AA. Priming showed little improvement in the banding pattern and intensity of protein in normal seeds, while low-vigor seeds had significant improvement in the banding pattern and intensity of protein.

Primed seeds under storage

Priming helps in premonsoon sowing prior to arrival of monsoon, the timing of priming is crucial. However, conducting the priming operation, monitoring water uptake and careful drying are difficult to manage in large scale especially at farmers level. Again Research has shown that primed seeds cannot be stored for more than few weeks due to reduced repair mechanisms present for DNA damage caused by progression in cell cycle during hydration (Van Pijlen *et al.*, 1996). When a primed seed is stored under conducive conditions (low temperature and low moisture) most of the beneficial effects of priming are retained. However, the storability of the primed seed per se is either improved or adversely affected, depending upon the initial physiological status of the seed.

Osmotic priming in a polyethylene glycol solution (300 g/kg water) for 48 h resulted in a partial loss of desiccation tolerance for seeds of *Vigna radiata* (L.) Wilczek (mung bean) (Sun *et al.*, 1997). He observed decrease in germination percentage of primed seeds when seeds were dried to water contents less than 0.06g/gDW. The decline of storage stability after osmotic priming was correlated by him with the modifications of seed water sorption properties. Priming significantly increased the amount of water associated with the weak water-binding sites, and reduced the amount of water associated with the strong binding sites and multi-molecular binding sites in seed tissues. The enhancement of molecular mobility in seeds, as a result of such water redistribution, probably accelerates seed deterioration and decreases storage stability.

Tarquis and Bradford (1992) also reported that while prehydration or priming treatments effectively accelerate subsequent germination rates of lettuce

seeds, the redried seeds are highly susceptible to deterioration in storage. Yeh (2005) recommended partial vacuum storage for extending the longevity of primed seeds. He observed improved seed germination, reduced lipid peroxidation and enhanced anti-oxidative activity prior to storage. However, primed seeds accumulated more total peroxide than non-primed control after 12 months non-vacuum storage, and this led to a marked decrease in seed longevity. Increased total peroxide levels were associated with decreased percentage of 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) radicle inhibition. He opined that improved longevity was related to enhanced anti-oxidative activity that minimized the accumulation of total peroxide during long-term storage. Chiu *et al.* (2003) also observed the beneficial effect of partial vacuum in storing the seeds of sweet corn.

Long storage periods of osmoprimed tomato seeds upto six months indicated that priming benefits could be maintained till two months and then started declining (Ismail *et al.*, 2005). Hsu *et al.* (2003) observed improved germination percentage and mean emergence time (MET) of bitter gourd seeds at sub-optimal temperature of 20°C with both priming (mixing seeds with moist vermiculite for 36 h at 20±8°C) and hot water soaking (soaking seeds for 4 h in water at 40±8°C) (a form of accelerated ageing). But the emergence percent decreased and MET increased by accelerated aging. He also observed increased lipid peroxidation with decreased activities of several free radical and peroxide scavenging enzymes.

Ways and means for assessing the quality of primed seeds

Moradi and Younesi (2009) used accelerated ageing test to determine the effectiveness of priming on sorghum seeds and observed that AA increased the Mean Germination Time and reduced emergence compared to control and opined that primed seeds were irreversibly damaged upon accelerated ageing.

Several researchers have reported the loss of phytates in primed seeds (Andriotis *et al.*, 2005 and Badau *et al.*, 2005). Estimation of phytic acid reduction could become a quality test for primed seeds in future. As DNA synthesis and rejuvenation forms a basis for increased cellular activity consequent to priming, measure of nuclear replication activity using flow cytometry will help to distinguish between well primed and poorly primed seeds.

Use of soft X ray for viewing embryo development in primed seeds and classification of primed seeds on basis of embryo enlargement and development will help in detection of primed vigorous seed. This technique could also be used to detect embryo damage upon priming and suggest suitable alternatives in priming technique.

Subjection of primed seeds to Seed Viability Equation

The Ellis-Robert's seed viability equation was used to predict seed survival after storage at specified temperatures and moisture contents. Seed priming, which can break dormancy and accelerate germination, can also reduce seed storage life. The priming effects on lettuce seeds in relation to the viability equation, were studied by Hill *et al.* (2007). However, they were not able to detect consistent differences in seed moisture absorption isotherms between non-primed and primed lettuce seeds. This was due to the exceedingly high moisture absorbed by seeds during accelerated ageing of already high moist primed lettuce seeds. Hence, it was recommended that a more accurate method for estimating relative deterioration of primed lettuce seeds may be to only increase storage temperature.

Future of priming

With a forecast of alarming climate change in future and loss of cultivable areas that are ravaged by the onslaught of unchallenged urbanization, farming will be pushed to its brink of arable lands. More lands will be converted into farm lands which will pose the twin problem of poor soil and high soil temperature. Seed quality will have to be manipulated by priming and will become a priority for future seeds men.

One problem though surmountable, still remains. It is the poor storability of primed seeds. Efforts will have to be made to determine the right time and right quantity of priming (time and solute infusion). Packing techniques have to be developed to store primed seed. Such techniques will revolutionize farming in moisture starved areas where farmers are poor technologically and traditionally.

More research has to be done in the area of using botanicals and use of microbial consortia for biopriming. With organic agriculture gaining prominence, such ventures will be economically and ecologically profitable.

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