The subcellular basis of seed priming

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Seed priming is a commercially used technique for improving seed germination and vigour. It involves imbibition of seeds in water under controlled conditions to initiate early events of germination, followed by drying the seed back to its initial moisture content. This review article summarizes the recent information available on the various subcelluar processes associated with priming which lead to seed enhancement. The paper discusses the role of synthesis of specific proteins in response to priming. The effect of priming on DNA repair, gene expression and synthesis of new message and protein synthesizing machinery are included. The enhancement in the energy metabolism of the cell by priming and the effect of priming in advancing and synchronizing the stage of the cell cycle are discussed. The article also includes information on the role of priming in specific cases, such as alleviation of dormancy in thermosensitive crops which require ethylene. The relationship between seed longevity and priming and methods to prolong longevity, wherever required after priming and the role of desiccation-related proteins, which accumulate during these treatments are also discussed. An illustration summarizing the information on all the metabolic processes which could possibly contribute towards the enhancement in seed performance achieved by priming is included. The paper identifies areas where information is lacking and potential for more in-depth research exists.

SEED priming is a technique which involves uptake of water by the seed followed by drying to initiate the early events of germination up to the point of radicle emergence[1–3]. Its benefits include rapid, uniform and increased germination, improved seedling vigour and growth under a broad range of environments resulting in better stand establishment and alleviation of phytochrome-induced dormancy in some crops. The techniques, which are commercially used to accomplish seed priming, have been reviewed in detail by Khan, Basu, McDonald, Copeland and McDonald, and Varier and Vari[2–6]. The common feature in these priming techniques is that they all involve controlled uptake of water. The metabolic processes associated with priming are slightly different, with respect to their dynamics from those which occur during germination, where the water uptake is not controlled. Also, the salts used during priming elicit specific subcellular responses.

Much of the earlier work on the physiology of seed priming has been reviewed by McDonald[3]. The goal of this article is to update the information on the subcellular basis of seed priming including recent references which

have not been covered by earlier reviews. In the discussion, we suggest our own model to summarize the various physiological processes which could possibly be involved in the improvements conferred by seed priming.

Stages of water uptake during germination where priming is relevant

When a dry seed is kept in water, the uptake of water occurs in three stages[7]. Stage I is imbibition where there is a rapid initial water uptake due to the seed's low water potential. During this phase, proteins are synthesized using existing mRNA and DNA and mitochondria are repaired. In stage II, there is a slow increase in seed water content, but physiological activities associated with germination are initiated, including synthesis of proteins by translation of new mRNAs and synthesis of new mitochondria. There is a rapid uptake of water in stage III where the process of germination is completed culminating in radicle emergence.

Stages I and II are the foundations of successful seed priming where the seed is brought to a seed moisture content that is just short of radicle protrusion[8,9]. The pattern of water uptake during priming is similar to that during germination but the rate of uptake is slower and controlled.

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Synthesis of proteins and enzymes during priming

A proteome analysis of seed germination during priming in the model plant *Arabidopsis thaliana* by MALDI-TOF spectrometry identified those proteins which appear specifically during seed hydropriming and osmopriming[10,11]. Among these are the degradation products of the storage protein 12S-cruciferin β-subunits. In a previous work on sugar beet, Job *et al.*[12] reported the accumulation of the degradation product of the B-subunit of 11-S globulin during seed priming by an endoproteolytic attack on the A-subunit. This suggests that enzymes involved in mobilization of storage proteins are either synthesized or activated during seed priming. Other reserve mobilization enzymes such as those for carbohydrates (α and β amylases) and lipids mobilization (isocitrate lyase) are also activated during priming[13,14]. These results indicate that priming induces the synthesis and initiates activation of enzymes catalysing the breakdown and mobilization of storage reserves, though most of the nutrient breakdown and utilization occur post-germinative after the radicle emergence.

The proteomic analysis also reveals that α and β tubulin subunits, which are involved in the maintenance of the cellular cytoskeleton and are constituents of microtubules involved in cell division, are abundant during priming. Accumulation of β-tubulins during priming has been observed in many species in relation with reactivation of cell cycle activity[15,16] and is discussed later.

Another protein detected by the proteomic analysis, whose abundance specifically increases during hydropriming, is a catalase isoform. Catalase is a free-radical scavenging enzyme. It is presumed that hydropriming initiates an oxidative stress, which generates reactive oxygen species, and catalase is synthesized in response to this stress to minimize cell damage. In addition to catalase, levels of superoxide dismutase, another key enzyme quenching free radicals also increases during priming[17,18]. Increased levels of these free radical scavenging enzymes due to the oxidative stress during priming could also protect the cell against membrane damage due to lipid peroxidation occurring naturally.

Shinde[19] reported synthesis of a 29 kD polypeptide after 2–6 h of priming in cotton seeds.

The abundance of low molecular weight heat shock proteins (LMW HSPs) of 17.4 and 17.7 kD specifically increased in osmoprimed seeds in the MALDI-TOF spectrometry analysis[10,11]. LMW HSPs are reported to have molecular chaperone activity[20], these data suggested that LMW HSPs may act by maintaining the proper folding of other proteins during osmopriming, preventing aggregation and binding to damaged proteins to aid entry into proteolytic pathways[21]. In osmopriming, seeds are soaked in osmotica, viz. polyethylene glycol (PEG) and mannitol, which result in incomplete hydration and an osmotic

stress situation is created. This explains the abundance of heat shock proteins, which are known to accumulate in high amounts during any kind of stress. These HSPs synthesized during osmopriming in response to stress could also protect the proteins damaged by natural ageing. Similarly, the enzyme L-isoaspartyl protein methyltransferase, which repairs age-induced damage to cellular proteins, is reported to increase in response to priming[22]. Thus, it appears that one of the ways in which priming is effective at the subcellular level is by conferring protection to the cellular proteins damaged through natural ageing.

Gene expression and synthesis of new mRNA during priming

Shinde[19] reported priming-induced synthesis of RNA in cotton seeds, corresponding to the actin 7 gene, following a reverse transcriptase polymerase chain reaction (PCR) analysis. Studies on gene expression in osmoprimed seeds of *Brassica oleracea* on a cDNA microarray[23,24] revealed that in primed seeds many genes involved in cellular metabolism are expressed (and synthesize mRNA) at a level intermediary between those in dry seeds and germinating seeds imbibed in water. These genes mostly code for proteins involved in energy production and chemical defence mechanisms. A few genes are expressed to the same extent in osmoprimed seeds as in germinating seeds. These include genes for serine carboxypeptidase (involved in reserve protein mobilization and transacylation) and cytochrome B (involved in the mitochondrial electron transport).

This microarray analysis in combination with Northern analysis gives some idea of transcripts synthesized during priming. To obtain direct evidence for the synthesis of new mRNA, techniques which involve detection of premature RNA species before intron splicing should be integrated with the other methods.

Effect of priming on protein synthesizing machinery

Priming improves the integrity of the ribosomes by enhancing rRNA synthesis[25]. The microarray gene expression studies of Soeda *et al.*[24] in *B. oleracea* seeds, reveal that RNA levels of genes encoding components of the translation machinery, such as ribosomal subunits and translation initiation and elongation factors, increase during osmopriming. Thus, one of the ways in which priming enhances protein synthesis is by improving the functioning of the protein synthesis machinery.

DNA repair during priming

Maintenance of the integrity of DNA by repairing the damages incurred naturally is important for generating

error-free template for transcription and replication with fidelity. Thornton et al.[26] suggested that damage to DNA which accumulates during the seed ageing is repaired by aerated hydration treatments as also during early hours of germination. DNA synthesis measured by the incorporation of ^{3}H thymidine in artificially aged seeds of B. oleracea L. was advanced by this treatment (compared to that in the untreated aged seeds) along with an improvement in germination. This recovery in DNA synthesis is attributed to pre-replicative repair of DNA damaged during ageing by the hydration treatment since treatment with hydroxyurea, which is an inhibitor of replicative DNA synthesis does not inhibit the synthesis. The exact mechanism of this repair is not yet known and needs to be investigated.

Association between priming and the cell cycle

To achieve maximum benefits from priming, the process is stopped just before the seed loses desiccation tolerance, i.e. before the radicle emergence or stage III of water uptake. Radicle emergence involves cell expansion and is facilitated by an increased turgor pressure in the hydrated seed, whereas active cell division starts after radicle emergence. So, it is expected that priming does not exert any major effect on cell division per se[27]. However, priming advances the cell cycle up to the stage of mitosis.

Flow cytometric analyses of osmoprimed tomato seeds reveal that the improvement of germination associated with priming is accompanied by increase in 4C nuclear DNA indicating that priming enhances DNA replication allowing the advancement of the cell cycle from G_1 to the G_2 phase[28]. Powell et al.[16] found an increase in the proportion of nuclear DNA present as 4C DNA in high vigour cauliflower seeds subjected to aerated hydration treatment. Thasni[29] also reported a two-fold increase in total genomic DNA content in hydro-primed corn seed.

Immunohistochemical labelling of DNA with bromodeoxyuridine (BrdU) during seed osmoconditioning in tomato confirms the presence of cells in the S-phase of the cell cycle synthesizing DNA[15]. The actively replicating DNA is tolerant to drying as incorporation of BrdU is detected in embryo nuclei before and after osmoconditioned seeds are re-dried. Although the frequency of 4C nuclei after the osmoconditioning treatment is higher than that of untreated seeds imbibed in water for 24 h, lower numbers of BrdU-labelled nuclei are detected in osmoconditioned embryos. This is because of the fact that though priming enhances DNA replication to some extent and facilitates the synchronization of DNA replication in all the cells of the embryo, DNA replication per se is lesser during priming under controlled hydration than during direct imbibition in water.

Powell et al.[16] following western analysis observed that the level of β-tubulin, which is a cytoskeletal protein and is related to the formation of cortical microtubules increases in response to aerated hydropriming. de Castro et al.[15] also observed accumulation of β-tubulin in all tissues of the tomato seed embryo during osmopriming. After redrying β-tubulin appeared as granules or clusters. This is because microtubules are sensitive to dehydration and are partly depolymerized after drying. The amount of soluble β-tubulin detected after re-drying is relatively high because microtubules are dynamic structures and exist in an equilibrium between soluble tubulin subunits and the polymerized microtubules. During priming, the cell cycle is arrested at the G_2 phase allowing the synchronization of cells. Mitotic events and cell division occur earlier and to a greater extent in embryos of primed seeds upon subsequent imbibition in water than in the control seeds. Thus, the pre-activation of the cell cycle is one of the mechanisms by which priming induces better germination performance relative to untreated seeds. The regulation of the cell cycle by priming could be through the regulation of the activity of the cell cycle proteins such as cyclins, cyclin dependent protein kinases and proliferating-cell nuclear antigens (PCNA). Imbibition of maize seed in the presence of benzyladenine increases the amount of PCNA over control, which is associated with the acceleration of the passage of cells from G_1 to G_2[30]. There is no information on the effect of priming on the cell cycle proteins and research needs to be initiated in this area.

Effect of priming on energy metabolism and respiration

Corbineau et al.[31] observed that imbibition of tomato seeds in PEG results in sharp increases in adenosine triphosphate (ATP), energy charge (EC) and ATP/ADP (adenosine diphosphate) ratio. These remain higher in primed seeds even after drying than in unprimed seeds. During subsequent imbibition in water, the energy metabolism of the primed and dried seed is much more than that of the unprimed seed making the primed seed more vigorous. The high ATP content of the redried primed seed is maintained for at least 4–6 months when stored at 20°C. Maximum benefit of osmopriming is obtained when performed in atmospheres containing more than 10% oxygen. Priming treatment is totally ineffective in the presence of the respiratory inhibitor (NaN₃) at high concentration, suggesting that respiration is essential for priming to be effective. The beneficial effect of priming is optimal for values higher than 0.75 for EC and 1.7 for the ATP/ADP ratio.

Hydropriming improves the integrity of the outer membrane of mitochondria after 12 h of imbibition (estimated by the cytochrome C permeation assay), but there is no concomitant increase in the ability of the mitochondria to oxidize substrates[32]. Significant increase in the

number of mitochondria in response to priming was also reported in osmoprimed leek cells[33], although these were not correlated to respiration levels. The association between improvement in the mitochondrial integrity by priming and mitochondrial performance needs to be elucidated.

Priming and seed 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[34,35]. The embryo in lettuce seed is enclosed within a two to four cell layer endosperm, whose cell walls mainly comprise galactomannan polysaccharides and hence the weakening of endosperm layer is a prerequisite to radicle protrusion, particularly at high temperatures. Endo-β-mannase is the key regulatory enzyme in endosperm weakening, which requires ethylene for activation. High temperatures reduce germination primarily through their inhibitory effect on ethylene production by seeds, which in turn reduces the activity of endo-β-mannase. Osmopriming of seeds with PEG (−1.2 MPa) at 15°C with constant light could overcome the inhibitory effects of high temperature in thermosensitive lettuce cv. Dark Green Boston (DGB) seeds in the absence of exogenous ethylene supply[36]. Imbibition of seeds of DGB in 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene) improved their germination at 35°C and also increases the activity of endo-β-mannase. Osmopriming of DGB seeds had a similar effect as imbibition in ACC, improving both germination and the activity of endo-β-mannase. This suggests that osmopriming is able to substitute the effect of ACC for breaking thermodormancy. Osmopriming in the presence of aminoethoxyvinylglycine (AVG), an inhibitor of ethylene synthesis (it inhibits ACC synthase) does not affect the enhancement of germination. Thus, osmopriming is able to overcome the dormancy even when ethylene synthesis is interrupted. A possible explanation for this is that osmopriming helps in releasing the ethylene within the embryonic tissues encased by the endosperm and seed coat and this would be sufficient to allow seed germination. Priming in the presence of silver thiosulphate (STS), a putative specific inhibitor of ethylene action, which interacts with the binding site of ethylene, inhibits germination, suggesting that ethylene activity is indispensable for the release of dormancy. There are several studies that show an increased ability for primed seeds to produce ethylene[35,37,38]. However, it is not clear whether ethylene production is integral to obtaining a priming effect in seeds or whether it is simply the result of high vigour displayed by primed seeds.

In other species also such as tomato, carrot and cucumber which do not require ethylene, priming enhances the loosening of the endosperm/testa region that permits germination at suboptimal temperatures[3].

Priming and seed longevity

In general, priming improves the longevity of low vigour seeds, but reduces that of high vigour seeds[2,39]. 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.

Powell et al.[16] observed that aerated hydration treatments improve storage potential of low vigour seeds and decrease the longevity of high vigour seeds. The improved longevity of low vigour seeds is associated with increased K_i (initial seed viability) after priming and a reduced rate of deterioration.

The most frequently cited cause of seed deterioration is damage to cellular membranes and other subcellular components by harmful free radicals generated by peroxidation of unsaturated and polyunsaturated membrane fatty acids. These free radicals are quenched or converted to less harmful products (hydrogen peroxide and subsequently water) by free radical scavenging enzymes and antioxidants. Hydropriming and ascorbic acid priming of cotton seed is reported to maintain germination and correspondingly the activities of a number of antioxidant enzymes such as peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase under artificial ageing condition[40]. Also the accumulation of by-products of lipid peroxidation, such as peroxides, malonaldehyde and hexanals is decreased by osmopriming, which is correlated with decreased loss in viability of soybean seeds under storage[41]. Solid matrix priming in moistened vermiculite reduces lipid peroxidation, enhances antioxidative activities and improves seed vigour of shrunken-2 sweet corn seed stored at cool or subzero temperatures[42]. Treatment of shrunken-2 sweet corn seeds with 2,2′-azobis 2-aminopropane hydrochloride (AAPH), a water-soluble chemical capable of generating free radicals, damages the seeds by increasing lipid peroxidation[43]. This damage is partially reversed by solid matrix priming which increases free radical and peroxide scavenging enzyme activity and subsequent reduction in peroxide accumulation.

As stated earlier, when high vigour seed lots are primed, their longevity gets adversely affected. Attempts have been made by several workers to develop methods to restore seed longevity after seed priming. Slow drying at 30°C which reduces the moisture of osmoprimed B. oleracea to 25% in the first 72 h of drying, followed by fast drying at 20°C to bring the moisture level down to 7% improved the performance of the osmoprimed seed in

a controlled deterioration test compared to that of the osmoprimed seed subjected to fast drying[24]. Concomitant with the improved longevity of slow dried-seeds is the enhanced expression of two stress tolerant genes during slow drying. These two genes namely *Em6* and *RAB 18*, which belong to the late embryogenesis abundant (LEA) protein groups, are also expressed to a large extent in mature seeds and are responsible for conferring desiccation tolerance during seed maturation. *Em6* belongs to group 1b LEA proteins and shares features with DNA gyrases or molecular chaperones which suggest a role for *Em6* in protecting DNA integrity during controlled deterioration treatments. *RAB 18* belongs to group 2 LEA proteins and encodes an abscisic acid (ABA)-inducible dehydrin. It accumulates in plants in response to drought stress and certainly has a protective role in stress tolerance but the exact mechanism is not known. These genes are expressed to a lesser extent in the fast dried seeds because the moisture content drops much too rapidly.

A post-priming treatment including a reduction in seed water content followed by incubation at 37°C or 40°C for 2–4 h restores potential longevity in tomato seeds[44,45]. This treatment is accompanied by the increase in the levels of the immunoglobulin binding protein (BiP) an ER resident homolog of the cytoplasmic hsp 70 (ref. 45). BiP is known to be involved in restoring the function of proteins damaged by any kind of stress and may function as a chaperone in the reactivation of proteins damaged due to the imbibition and drying processes involved in seed priming.

Discussion and conclusions

Pre-sowing priming improves seed performance as the seed is brought to a stage where the metabolic processes are already initiated giving it a head start over the unprimed seed. Upon further imbibition, the primed seed can take off from where it has left completing the remaining steps of germination (stage III) quicker than the unprimed seed. Priming also repairs any metabolic damage incurred by the dry seed, including that of the nucleic acids, thus fortifying the metabolic machinery of the seed. Another beneficial effect of priming is the synchronization of the metabolism of all the seeds in a seed lot, thus ensuring uniform emergence and growth in the field.

The different ways in which priming could possibly be effective at the subcellular level in improving seed performance is depicted in Figure 1. This figure is an adaptation of the figure suggested by Bewley *et al.*[7] to illustrate the metabolic events in the seed upon imbibition in water. Since hydration is also the key process in priming, albeit in a controlled fashion, and conforms to the triphasic pattern of water uptake, the original figure has been superimposed with the present one to describe the subcellular events specifically associated with priming. The figure also incorporates other aspects of priming discussed in the earlier sections such as its effect on dormancy release and seed longevity.

The most important ameliorative effect of priming should be the repair of damaged DNA to ensure the availability of error free template for replication and transcription. Since the water uptake is slower during priming than germination, the seed gets more time for completion of the process of repair. Unfortunately, there is no direct experimental evidence to support or corroborate this. One strategy (there could be other possible approaches) to specifically detect repair synthesis differentiating it from replicative synthesis is to artificially induce damage to DNA of the seed by UV irradiation. The damaged seeds can then be primed, the DNA labelled with BrdU, and ssDNA transients generated during repair in response to priming can be detected using an anti-BrdU antibody.

It is evident that priming advances the metabolism of the seed. Many proteins and enzymes involved in cell metabolism are synthesized to a level intermediary between the dry seed and the seed imbibed directly in water, while a few of these are synthesized to the same extent as the germinating seed.

Some proteins are synthesized only during priming and not during germination. For example, 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 osmopriming) can induce the breakdown of these proteins thus initiating the process of reserve protein mobilization earlier than in the unprimed seed. Similarly, low molecular weight HSPs are specifically synthesized during osmopriming and not during imbibition in water. These proteins function as molecular chaperones and are synthesized to protect the cell from moisture stress occurring during the process of osmopriming but they could very well be effective in protecting those proteins also which are damaged naturally. Free radical scavenging enzymes such as catalase and superoxide dismutase are synthesized during hydropriming to protect the cell from damage due to lipid peroxidation, which occurs due to the oxidative stress induced by hydropriming. These enzymes could also be effective in quenching the free radicals generated by lipid peroxidation occurring naturally.

Priming synchronizes all the cells of the germinating embryo in the G_2 phase of the cell cycle so that upon further imbibition, cell division proceeds uniformly in all the cells ensuring uniform development of all parts of the seedling. 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. There is enhanced ATP production during
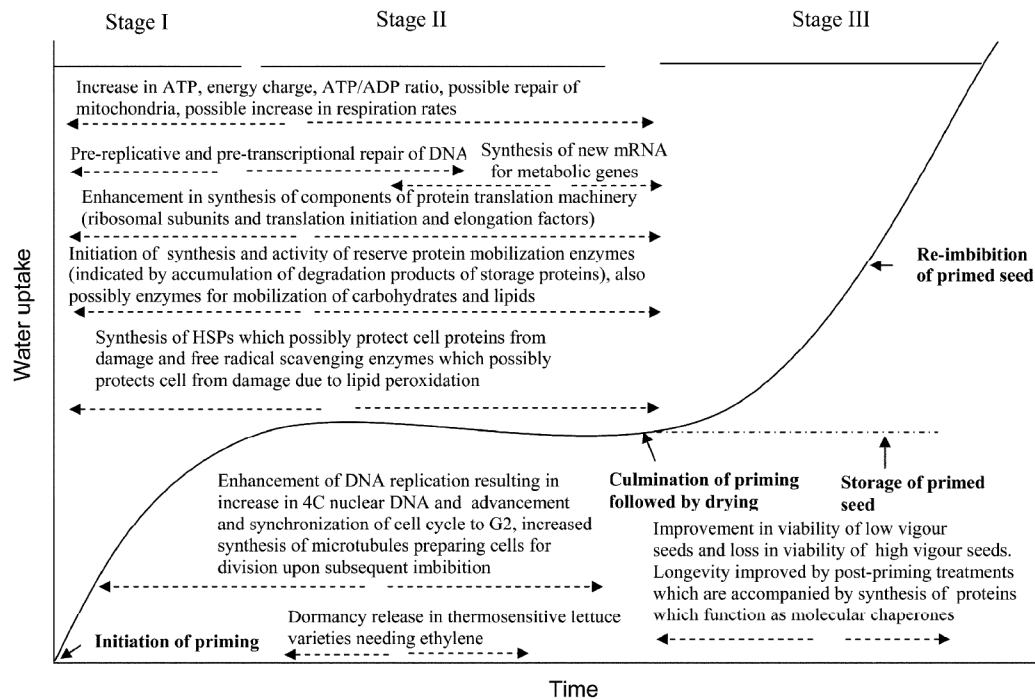
Figure 1. The pattern of water uptake and metabolic events during seed priming.

priming, which is retained even after drying making the primed seed more vigorous than an untreated seed.

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. Priming improves the storability of low vigour seeds, but reduces that of high vigour seeds. The longevity of seeds after priming can be extended by giving post-priming treatments involving subjecting the seed to slight moisture and temperature stress before drying the seed completely. These treatments are accompanied by the synthesis of stress related proteins (similar to those which are abundant when the seed undergoes desiccation during maturation) which protect the cellular proteins from damage and thus, in turn, extend the seed longevity.

While we know that all the beneficial subcellular responses induced by seed priming occur between stages I and II of water uptake, we are not able to give the exact sequence of their occurrence at this point in time. Similarly, for optimization of priming technology, no suitable marker is reported, which can indicate the completion of stage II. This can be of immense practical use. More in-depth research on the physiology of seed priming would help us to refine the technique and develop better priming protocols to achieve maximum benefits.

1. Matthews, S. and Powell, A. A., Seed treatments: developments and prospects. *Outlook Agric.*, 1988, **17**, 97–103.

2. Basu, R. N., An appraisal of research on wet and dry physiological seed treatments and their applicability with special reference to tropical and subtropical countries. *Seed Sci. Technol.*, 1994, **22**, 107–126.

3. McDonald, M. B., Seed priming. In *Seed Technology and its Biological Basis* (eds Black, M. and Bewley, J. D.), CRC Press, Florida, 2000, pp. 287–325.

4. Khan, A. A., Preplant physiological seed conditioning. In *Horticultural Reviews* (ed. Janick, J.), John Wiley and Sons, New York, 1992, vol. 14, pp. 131–181.

5. Copeland, L. O. and McDonald, M. B., Seed enhancements. In *Principles of Seed Science and Technology* (eds Copeland, L. O. and McDonald, M. B.), Kluwer Academic, Dordrecht, 2001, pp. 277–296.

6. Varier, A. and Vari, A. K., Seed enhancement technology. In *Seed Science and Technology* (ed. Singhal, N. C.), Kalyani Publishers, New Delhi, 2010, pp. 182–198.

7. Bewley, J. D., Seed germination and dormancy. *Plant Cell*, 1997, **9**, 1055–1066.

8. Akers, S. W. and Holley, K. E., SPS: a system for priming seeds using aerated polyethylene glycol or salt solutions. *Hortic. Sci.*, 1986, **21**, 529–531.

9. Bray, C. M., Biochemical processes during osmoconditioning of seeds. In *Seed Development and Germination* (eds Kigel, J. and Galli, G.), Marcel Dekker, New York, 1995, pp. 767–789.

10. Gallardo, K., Job, C., Groot, S. P. C., Puype, M., Demol, H., Vandekerekhove, J. and Job, D., Proteomic analysis of *Arabidopsis* seed germination and priming. *Plant Physiol.*, 2001, **126**, 835–848.

11. Gallardo, K., Job, C., Groot, S. P. C., Puype, M., Demol, H., Vandekerekhove, J. and Job, D., Proteomics of *Arabidopsis* seed germination and priming. In *The Biology of Seeds: Recent Advances* (eds Nicholas, G. *et al.*), CABI, Cambridge, 2004, pp. 199–209.

12. Job, D., Capron, I., Job, C., Dacher, F., Corbineau, F. and Come, D., Identification of germination-specific protein markers and their use in seed priming technology. In Seed Biology Advances and Applications: Proceedings of the Sixth International Workshop on

Seeds (eds Black, M., Bradford, K. J. and Vasquez-Ramos, J.), Merida, Mexico, 1999, CABI, Cambridge, 2000, pp. 449–459.

13. Fu, J. R., Lu, X. H., Chen, R. Z., Zhang, B. Z., Liu, Z. S. and Cai, C. Y., Osmoconditioning of peanut (*Arachis hypogea* L.) seeds with PEG to improve vigour and some biochemical activities. *Seed Sci. Tech.*, 1988, **16**, 197–212.

14. Sung, F. J. M. and Chang, Y. H., Biochemical activities associated with priming of sweet corn seeds to improve vigor. *Seed Sci. Tech.*, 1993, **21**, 97–105.

15. de Castro, R. D., van Lammeren, A. A. M., Groot, S. P. C., Bino, R. J. and Hilhorst, H. W. M., Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. *Plant Physiol.*, 2000, **122**, 327–335.

16. Powell, A. A., Yule, L., Jing, H. C., Groot, S. P. C., Bino, R. J. and Pritchard, H. W., The influence of aerated hydration seed treatment on seed longevity as assessed by the viability equations. *J. Exp. Bot.*, 2000, **51**, 2031–2043.

17. Bailly, C., Benamar, A., Corbineau, F. and Come, D., Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Sci. Res.*, 2000, **10**, 35–42.

18. Vari, A., Mitrabinda, S., Dadlani, M. and Sharma, S. P., Physiological and biochemical changes associated with osmopriming in maize seeds, Paper presented in II International Congress of Plant Physiology, 8–12 January 2003, New Delhi, p. 113.

19. Shinde, P. Y., Evaluation and enhancement of seed quality in cotton, Ph D thesis submitted to IARI, New Delhi, 2008, p. 106.

20. Lee, G. J., Pokala, N. and Vierling, E., Structure and *in vitro* molecular chaperone activity of cytosolic small heat shock proteins from pea. *J. Biol. Chem.*, 1995, **270**, 10432–10438.

21. Nover, L. and Scharf, K. D., Heat stress proteins and transcription factors. *Cell Mol. Life Sci.*, 1997, **53**, 80–103.

22. Kester, S. T., Geneve, R. L. and Houtz, R. L., Priming and accelerated ageing effect L-isoaspartylmethyltransferase activity in tomato (*Lycopersicon esculentum* Mill.) seed. *J. Exp. Bot.*, 1997, **48**, 943–949.

23. Groot, S. P. C., Soeda, Y., Stoopen, G., Konings, C. J. M. and van der Geest, A. H. M., Gene expression during loss and regaining of stress at seed priming and drying. In *The Biology of Seeds: Recent Advances* (eds Nicholas, G. *et al.*), CABI, Cambridge, 2004, pp. 279–287.

24. Soeda, Y. *et al.*, Gene expression programs during *Brassica oleracea* seed maturation, osmopriming and germination are indicators of progression of the germination process and the stress tolerance level. *Plant Physiol.*, 2005, **137**, 354–368.

25. Coolbear, P., Slater, R. J. and Bryant, J. A., Changes in nucleic acid levels associated with improved germination performance of tomato seeds after low-temperature presowing treatment. *Ann. Bot.*, 1990, **65**, 187–195.

26. Thornton, J. M., Collins, A. R. S. and Powell, A. A., The effect of aerated hydration on DNA synthesis in embryos of *Brassica oleracea* L. *Seed Sci. Res.*, 1993, **3**, 195–199.

27. Gurusinghe, S., Cheng, Z. and Bradford, K. J., Cell cycle activity is not essential for germination advancement in tomato. *J. Exp. Bot.*, 1999, **50**, 101–110.

28. Ozbingol, N., Corbineau, F., Groot, S. P. C., Bino, R. J. and Come, D., Activation of the cell cycle in tomato (*Lycopersicon esculentum* Mill.) seeds during osmoconditioning as related to temperature and oxygen. *Ann. Bot.*, 1999, **84**, 245–251.

29. Thasni, K. A., Seed quality attributes and their enhancement in quality protein maize, M Sc thesis submitted to IARI, New Delhi, 2003, p. 86.

30. Sanchez, M. D. L., Gurusinghe, S. H., Bradford, K. J. and Vazquez-Ramos, J. M., Differential response of PCNA and Cdk-A proteins and associated kinase activities to benzyladenine and abscisic acid during maize seed germination. *J. Exp. Bot.*, 2005, **56**, 515–523.

31. Corbineau, F., Ozbingol, N., Vineland, D. and Come, D., Improvement of tomato seed germination by osmopriming as related to energy metabolism. In Seed Biology Advances and Applications: Proceedings of the Sixth International Workshop on Seeds (eds Black, M., Bradford, K. J. and Vasquez-Ramos, J.), Merida, Mexico, 1999, CABI, Cambridge, 2000, pp. 467–474.

32. Benamar, A., Tallon, C. and Macherel, D., Membrane integrity and oxidative properties of mitochondria isolated from imbibing pea seeds after priming or accelerated ageing. *Seed Sci. Res.*, 2003, **13**, 35–45.

33. Ashraf, M. and Bray, C. M., DNA synthesis in osmoprimed leek (*Allium porrum* L.) seed. *Seed Sci. Res.*, 1993, **3**, 15–23.

34. Nascimento, W. M., Cantliffe, D. J. and Huber, D. J., Ethylene evolution and endo-β-mannase activity during lettuce seed germination at high temperature. *Sci Agric.*, 2004, **61**, 156–163.

35. Cantiffe, D. J., Nascimento, W. M., Sung, Y. and Huber, D. J., Lettuce endosperm weakening: a role for endo-β-mannase in seed germination at high temperature. In Seed Biology Advances and Applications: Proceedings of the Sixth International Workshop on Seeds (eds Black, M., Bradford, K. J. and Vasquez-Ramos, J.), Merida, Mexico, 1999, CABI, Cambridge, 2000, pp. 277–284.

36. Nascimento, W. M., Cantliffe, D. J. and Huber, D. J., Thermotolerance in lettuce seeds: association with ethylene and endo-beta-mannanase. *J. Am. Soc. Hortic. Sci.*, 2000, **125**, 518–524.

37. Habdas, H., Szafirowska, A. and Sokolowska, A., Cytological and physiological effects of matriconditioning on low viable cucumber seed germination. *Acta Hortic.*, 2000, **517**, 113–120.

38. Siriwitayawan, G., Dutt, M., Kester, S., Downie, B. and Geneve, R., Ageing in tomato reduces the capacity of seeds to produce ethylene, while priming increases ethylene evolution during germination. In *The Biology of Seeds: Recent Advances* (eds Nicholas, G. *et al.*), CABI, Cambridge, 2004, pp. 441–446.

39. Pandita, V. K. and Nagarajan, S., Osmopriming of fresh seed and its effect on accelerated ageing in Indian tomato (*Lycopersicon esculentum*) varieties. *Indian J. Agric. Sci.*, 2000, **70**, 479–480.

40. Goel, A., Goel, A. K. and Singh, S. I., Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J. Plant Physiol.*, 2003, **160**, 1093–1100.

41. Braccini, A. L. E., Reis, M. S., Moreira, M. A., Sediyama, C. S. and Scapim, C. A., Biochemical changes associated with soybean seeds osmoconditioning during storage. *Pesq. Agropec. Bras.*, 2000, **35**, 433–447.

42. Chiu, K. Y., Chen, C. L. and Sung, J. M., Effect of priming temperature on storability of primed *sh-2* sweet corn seed. *Crop Sci.*, 2000, **42**, 1996–2003.

43. Sung, J. M. and Chiu, K. Y., Solid matrix priming can partially reverse the deterioration of sweet corn seeds induced by 2,2′-azobis(2-amidinopropane) hydrochloride generated free radicals. *Seed Sci. Technol.*, 2001, **29**, 287–298.

44. Gurusinghe, S. and Bradford, K. J., Galactosyl-sucrose oligosaccharides and potential longevity of primed seeds. *Seed Sci. Res.*, 2001, **11**, 121–133.

45. Gurusinghe, S., Powell, A. L. T. and Bradford, K. J., Enhanced expression of BiP is associated with treatments that extend storage longevity of primed tomato seeds. *J. Am. Soc. Hortic. Sci.*, 2002, **127**, 528–534.