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Seed Priming and Fortification

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ABSTRACT Seed priming is an essential pre-requisite for rapid, uniform germination of seeds and optimum plant stand in the field. The seed quality enhancement through seed priming has led to improvements in growers' ability to achieve better plant stand both in the field and greenhouse. During seed priming, the water content of the seed increases up to 35-40 per cent of its weight, enough to activate the biochemical events, advancing seed germination processes without radicle emergence. The product of these changes persist following desiccation and are available on re-imbibition of water, during seed planting, enabling completion of seed germination rapidly, led to an uniform crop stand and synchronized flowering/ fruiting. Additionally, this physiological treatment induces tolerance to certain environmental stresses (Like high temperature, low soil moisture etc), and provide invigoration treatment to partially aged seed lots. Under invigoration, metabolic repair processes in deteriorated seeds occur before onset of seed germination process. Priming can be achieved in several ways namely, imbibition in an osmotic solution [Poly ethylene glycol (PEG) or manitol] that control the water uptake (Osmo-conditioning), slow addition of water to bring the seeds to a specific water content (Hydro-priming) or mixing the seeds with a solid matrix materials like, vermiculite or diatomaceous earth and water (Solid matrix priming). If the priming solution is fortified with growth promoting substances or beneficial micro-organisms, which accelerate the germination or control the disease proliferation termed as bio-priming. The key processes involved in seed priming are, the early onset of RNA, protein synthesis and polyribosome formation. The activity of many enzymes involved in mobilization of storage reserve is triggered or *de novo* synthesis of important enzymes involved in pre-germination events occurs. On-farm priming is performed by soaking the seeds in water overnight, surface dried and planting the seeds on the same very day. If sowing is delayed, the seeds can be re-dried and stored, and planted as and when required. Significant yield improvements had been reported by various workers for a number of crops. The present paper discusses various methods of seed priming, its advantages, the physiological and biochemical changes involved and precautions to be observed.

Key words: Seed priming, fortification, hydro priming, solid matrix priming, vigour, longevity.

Improving the quality of seeds is an approach which is likely to produce significant benefits in almost all circumstances without any significant increase in risk. The most valuable gains in establishment are likely to come from improving seed quality and then integrating these benefits with the improvements that can be gained from other cultural practices.

Seed germination enhancement technologies based on pre-sowing seed hydration have attracted considerable interest in both seed physiological research and seed industry, where they have been commercialized. The use of seed enhancement technologies is not new to agriculture and earlier practices have been described for such treatments. Theophrastus (372-287BC) has recommended the presoaking of cucumber seeds in milk or water to make them germinate quicker and better. Oliver de Serres as early as 1600 has brought out the uses of seed

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soaking in water fertilized with best manure and dried back in shade. Another description from the same text illustrates the use of sowing partially dried seed which would emerge quickly avoiding the danger of being eaten away by soil pests or delayed by unfavourable weather conditions.

In modern agriculture however, Heydecker's work [1] is often taken as the starting point for pre-sowing seed treatments. By manipulating water relations to exploit most seed's natural ability to survive one or more cycles of imbibition and drying, subsequent germination is made faster and more uniform which Heydecker distinguished as the advancement and priming response, respectively. In recent years, however the term priming is commonly used to describe seed pre-sowing hydration methodologies without any discrimination, as to how and where seeds are imbibed and by whatever means [2-5].

Priming is a process in which seeds are imbibed either in water or osmotic solution or a combination of solid matrix carrier and water in specific proportions followed by drying before radicle emergence.

METHODS OF SEED PRIMING

Fundamentally, three strategies are used to deliver and restrict the amount of water and these are; submersion in solutions of osmotica in water, mixing with moist solid particulate material and hydration with water only. The extensive reviews by Welbaum and co-workers [6] and Mc Donald [7] provide useful information on the recent modifications of these techniques and the crops in which these methods have been successful.

Osmopriming

Osmotic priming of seeds also known as osmopriming or osmoconditioning describes incubation of seeds with aerated solutions of low water potential which are rinsed off afterwards. Mannitol or inorganic salts such as those of potassium or calcium (Chloride, nitrate, dihydrogen phosphate) have been used extensively as osmotica but because of their low molecular size these are capable of being absorbed by the seeds. In some cases this is associated with toxic side effects. Heydecker and co-workers [1] first suggested the use of moderately high molecular weight polymers such as poly ethylene glycol (6-8 Kda) whose large size precludes it from entering the seeds and since then it has become the most common inert osmoticum used for osmoconditioning by researchers and seed industry. Care must be taken to ensure adequate gas exchange by constant vigorous aeration or using stirred bioreactors [7]. Some seeds particularly onion osmo-primed satisfactorily only in air enriched with oxygen. Rowse and Mckee [8] introduced an improved version of osmopriming using semi-permeable membrane which they called membrane priming where the seeds are separated from the osmoticum contained in the outer jacket of a rotating tube. This device works better for small seeded flower species and for seeds with mucilaginous coats that can cause difficulties in other priming methods.

Hydropriming

Priming in pure water is termed hydropriming. Here water uptake can be either controlled or non-controlled. Non-controlled water uptake includes those methods in which water is available freely to the seeds and not restricted by the environment. Therefore water uptake is governed by the affinity of the seed tissues for water. Common techniques include imbibing seeds on moistened blotters or soaking seeds in water. Soaking by submerging seeds in water can be performed with or without aeration [9]. Since water is not limited, seeds germinate if they are viable and non-dormant, so also depending on the availability of oxygen and a suitable temperature. Therefore the process must be arrested at a specific time in non-controlled water uptake systems to prevent the onset of phase III of germination - water uptake pattern is generally triphasic with an initial rapid uptake known as imbibition (Phase I) followed by a lag phase (Phase II) and then the second rapid increase in water uptake resulting in radicle emergence (Phase III) [10]. Seeds are desiccation tolerant during the first two phases but become

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desiccation sensitive during phase III. Each phase of water uptake is controlled by water available to the seeds.

In controlled water uptake, water availability or water potential is regulated thus preventing the completion of germination. Water potential is the algebric sum of osmotic, matric and pressure potentials. In hydro priming, controlled hydration is achieved by adding calculated amount of water to a given seed sample of known weight and moisture content. On a commercial scale, controlled hydration by drum priming elevates seeds to the desired moisture levels by applying given amount of water with time [11]. The duration of drum priming depends on the absorptive characteristics of the particular species and seed lot and on the desired seed moisture content of the finished product [12]. In one type, the priming drum is placed on the top of a scale which determines the seed weight increase created by a constant influx of water. The scale must be sensitive enough to determine small increments in weight caused by the addition of water in comparison with the weight of the apparatus itself. After the seeds have achieved the desired weight the hydration phase is complete. Hydrated seeds can be incubated for a desired period required of priming. Another method of drum priming system controls the seed hydration by the time interval and volume of water application [13]. A preset volume of water is injected during each cycle as regulated by a timer attached to a solenoid valve. Dividing the total volume of water required by the preset water injection volume establishes the number of cycles of injections. The water absorption pattern of a particular species and cultivar must be known so that the time set between water injections allows no free water to be left before the next water injection. After the seeds have attained the desired water content, they can be maintained in this condition, for a specific period to complete the priming process.

Solid Matrix Priming or Matriconditioning

This is a method more recently introduced where the seeds are soaked in an aqueous suspension of such solid materials, as ground lignite coal substances, vermiculate, press mud, diatomaceous earth, hydrous silicate clay or calcined clay. The quantity of solid particulate required per given mass of seeds is dependent on the water holding capacity of the material and the ability to remain friable throughout the process. Therefore the solid particulate acts as a means initially to hold large quantities of water releasing it slowly to the seeds when the latter is incubated with the moist carrier. The proportion of seed to solid particulate matter to water is determined on an empirical basis. Seeds slowly imbibe to reach an equilibrium hydration level determined by the reduced matrix potential of water adsorbed on the particle surfaces, and after the incubation, the moist solid material is removed by sieving. In this approach, adequate aeration is ensured to prevent the formation of temperature gradient in the seed mass. The surplus matrix can be removed without mechanically damaging the seed or leaving too much of dust on it.

Biopriming

Several researchers have investigated the use of beneficial microorganisms in the priming medium to control disease proliferation during priming itself. Warren and Bennet [14] added *Pseudomonas aureofaciens* as a biological control organism in combination with osmopriming treatment to control *Pythium ultimum* in tomato seedlings. Similar effects can be obtained by the other priming methods.

Besides biopriming, many studies reported the benefits of fortification of the priming medium with gibberellins, ethylene, cytokinins such as benzyl adenine. Adding such plant growth regulators during priming improve the germination performances of some species or seed lots. Addition of promotive compounds to seeds during priming can be advantageous in other ways too. Growth retardants have been advocated to dwarf the growth habit of transplants such as bedding plants which tend to develop an etiolated growth habit if grown in low light environment. Machado and co-workers [15] reported that priming of tomato cultivars with a triazole produced shorter green and more uniform seedlings with stronger and thicker stems and higher root shoot ratios than non-primed

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controls. Pill and Gunter [16] found similar dwarfing response in marigold seeds matriconditioned with paclobutrazol. Adding GA_3 to soybean seeds during osmoconditioning improved percentage germination and emergence. Lorenz & co-workers [17] and Bradford & co-workers [18] combined seed priming fungicides and anticrustants to hybrid muskmelon seeds and achieved earlier emergence and adequate plant densities for acceptable yield. Parera and Cantliffe [3] reported that Shrunken-2 sweet corn seeds had improved cold test performance, if primed with sodium hypochlorite (Solid matrix priming reduced fungal invasions).

On-farm Priming

Dr. Dave Haris, since 1996, had led a multi country research group to develop test and promote on-farm seed priming. This is a simple technique, in which seeds are soaked in water before sowing and results in average yield increase of around 30 per cent in many crops. This method has been adopted by thousands of resource-poor farmers for many crops *eg.*, upland rice, maize, wheat, barley, sorghum, pearl millet, finger millet, chickpea, mungbean, cowpea etc. in many countries in both Asia and Africa. Recent research has shown that seed priming can be used to increase yield further, about 30 per cent by overcoming soil micro and macro nutrient deficiencies and improving disease resistance. Research in eastern India, Nepal and Bangladesh has shown that adding rhizobia to the water used to prime legumes is as effective as using more expensive and complicated seed coating methods, and easy to be adopted by farmers. In Pakistan, maize seeds soaking, prior to sowing, in a solution of phosphate produced 24 per cent more grain than non-primed crop. The cost per hectare of the additional phosphate is negligible. Results showed that priming with tiny amounts of phosphate solution can substitute substantial amounts of phosphate fertilization for resource poor farmers, particularly in Africa where soil is a major constraint for crop growth. In the acidic soils, widely spread over eastern India, Nepal, west Banagladesh and many parts of east Africa, where poor legume growth is registered due to non-availability of molybdenum. In the soil, addition of salts, such as sodium molybdate, is expensive and is rather difficult to spread small amounts in large areas. Substantial yield benefits of 20-90 per cent can result from the tiny amounts of molybdate added to priming water. Costs are negligible and this single approach has been adopted by thousands of farmers, who otherwise were not able to grow a predictable crop of chickpea.

Farmers can prime their own seed, if they know the safe limit, the maximum time for which seeds are soaked, and which if exceeded could lead to seed and seedling damage. These limits are calculated for each variety so that germination will not continue once seeds are removed from the water. In most cases seeds can be primed over night, surface dried and sown on the same day. If primed seed is surface dried and kept dry, it can be stored for several days; sown as usual and still perform better than non-primed seeds. Research suggests that some of the efforts of on-farm seed priming can be gained by more sophisticated seed bed preparation and sowing methods. For example, mechanical plantation that ensures good seed-soil contact encourages good establishment. The results of over a thousand on-farm trials in paired plots for maize, rice, chickpea and wheat show that seed priming is good insurance for the farmer. There have been almost no negative effects on the crops; in some cases no effect at all has been observed, and whereas, in most cases there have been profound benefits.

Participation in technology development through the Indo-British rain fed farming project in parts of Gujarat, Madhya Pradesh and Rajasthan has empowered farmers to the extent that the farmers in many villages, within three years of exposure to the technology of a participatory approach, no longer use dry seed but use only primed seeds of maize, rice and chickpea with excellent results.

BENEFITS OF SEED PRIMING

The benefits of seed priming have been well documented in several reviews [2,4&19]. Seeds are primed for the following reasons:

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1) To overcome or alleviate phytochrome induced dormancy (Lettuce and celary), to decrease the time necessary for germination and subsequent emergence. To improve the crop stand uniformity, in order to facilitate production, management and enhance synchronisation at flowering. From a mechanical point of view, priming enables seeds of several species to germinate and emerge at supra-optimal temperature, increase in speed of germination and uniform field emergence.

2) Emergence occurs before soil becomes fully detrimental.

3) Crops compete more efficiently with weeds.

4) Increased control can be exercised over water usage and scheduling.

5) Priming has been commercially used to eliminate or to greatly reduce the amount of seed-borne fungi and bacteria. Organisms such as *Xanthomonas campestris* in *Brassica* seed and *Septoria* in celary had shown to be eliminated within a seed lot as a by-product of priming.

Priming is often used as a seed invigoration treatment for improving the germination and vigour in low vigour seed-lots. Hence it appears to reverse the detrimental effects of seed deterioration *i.e.*, repair of DNA and protein synthesising machinery.

CONDITIONS FOR PRIMING

The success of priming is variable and dependent on several factors. The initial seed viability or vigour has a definite bearing on the success of priming [20]. In pepper, osmoconditioning improves the performances of seed lots having a high percentage of viable seeds. Passam and co-workers [21] and Penaliza and Eira [22] found that hydration-dehydration treatments improved the germination of medium quality (Not high quality) tomato seeds, subjected to accelerated ageing for 72 hr at 100 per cent RH and 42°C. In cereals, seed germination and vigour were improved by priming high quality seeds [23]. In contrast, Burgeus and Powell [24] reported that high vigour brussel sprout seeds showed the least and low vigour seeds the most response to seed priming. The same was true for carrot, leek and onion seeds. Welbaum and Bradford [25] showed that the success of priming was dependent on seed maturity in muskmelon. Seeds harvested after 40 days from anthesis were more responsive than those harvested at 60 days.

The ideal priming method, moisture and oxygen conditions necessary for optimum priming responses are debatable. Smith and Cobb [26] concluded that the priming response was dependent on the duration and osmotic potential of the solutions. Salt solutions successfully primed tomato and carrot seeds the percentage emergence of treated onion seeds considerably reduced [27]. Rivas and co-workers [28] found that KNO_3 primed Jalapeno pepper seeds had earlier germination and accelerated vegetative growth, whereas, PEG primed seeds had retarded vegetative development. Osmoconditioning was not beneficial to flatpea seed performance [29].

Seed moisture content has an important bearing on priming success. In lettuce, priming shifted the maximum temperature at which 50 per cent of the seeds germinated to a higher value. Use of PEG prior to planting reduced the incidence of imbibitional injury common to Shruken-2 sweet corn [30]. Ellis and co-workers [31] suggested that very dry seeds should be pre-humidified to 9-10 per cent moisture content to avoid imbibitional damage. Chowdhury and Choudhuri [32] reported that pre soaking of jute seeds for 3-6 hr followed by dehydration than those for longer duration (12-24 hr) had the least damage on germination and early seedling growth.

The oxygen content during priming influences subsequent germination and seedling development. Bujalski and co-workers [33] and Bujalski and Nienow [34] reported that using enriched air with 75 per cent O and 25 per cent N ratio to aerate onion seeds in PEG solution provided the same number of normal seedlings as untreated seeds germinated on filter paper. But priming of muskmelon seeds in water under high oxygen content (>50%) decreased germination [35]. As a seed lot becomes over-

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primed, due to too long an exposure at the chosen water potential and temperature, more and more of individual seeds typically display damaged primary radicle meristem and the seedlings may not develop properly. Even though only a small per cent of seeds in a lot may be affected, such losses are unacceptable for high value seeds in commercial practice. It is therefore valuable to determine the safe limits for priming to minimize and preferably avoid these handicaps.

POST PRIMING PROCESSING

Post treatment processing including drying and storage of primed seeds has received little attention; this is a significant oversight as the beneficial effects of priming can be lost by improper post treatment handling [4]. The rate of drying after priming plays a significant role in maintaining the benefits derived through priming. The effect of time and extent of drying has been studied on imbibed perennial rye grass (*Lolium perene*) seeds [36]. Primed seeds when dehydrated at -150Mpa (32% RH) lost the gains accrued during priming *i.e.*, a loss in uniformity of germination was measured whereas, if the same seeds were initially dried at higher water potential of –4 Mpa (97% RH) for 24 hr before drying at 32 per cent RH. Similar findings were reported for carrot and pepper seeds [37]. When hydrated seeds were dried at different rates over saturated salt solutions and tested for germination there was delayed germination, and the germination advancement associated with hydration treatment was completely eliminated. In contrast, tomato seeds were largely unaffected by drying rates. Using *in situ* infrared microspectroscopy, Wolkers and co-workers [38] found that fast drying resulted in weak strength of hydrogen bonding at room temperature, a less clearly defined glassy matrix, apparently less tight molecular packing and greater extent of protein denaturation.

LONGEVITY OF PRIMED SEEDS

Primed seeds often exhibit reduced longevity during storage particularly under adverse storage conditions [39, 40]. In some seeds even relatively short hydration periods which do not advance germination can dramatically reduce seed longevity [41]. The storage environment has a profound effect on primed seed efficacy and longevity. Results of longevity after priming are inconsistent which may be partially attributed to the storage conditions (Temperature, RH/ moisture content and time of storage). Storage studies were performed on primed and non-primed tomato seeds aged at 42°C and 92 per cent RH for 15 days [42]. Prior to ageing, seeds germinated faster than non-primed seeds; however no differences in germination rate were measured between treatments aged for 6 days. If aged for more than 6 days, primed seeds performed poorly as compared to non-primed (Control) seeds. Here 6 days of ageing was considered the transition point between the beneficial effects of priming and deleterious effects of ageing. These conditions are crop and species specific and hence blanket rules cannot be followed. Efficient methods to predict seed lot performance to priming and dehydration required a series of empirical treatments. Recently, various treatments imposed after priming but before dehydration have been reported to improve seed longevity in storage [43]. The most effective treatments included both a moderate reduction in seed moisture contents and short periods of incubation at elevated temperature [41, 43 & 44]. Seed longevity in storage was reduced by rapid dehydration following a period of hydration. A reduction in seed moisture content or exposure to elevated temperature after priming might induce some physiological or molecular changes that would render the seeds more resistant to deterioration during storage.

Physiological, Biochemical and Molecular Changes Occurring during Seed Priming and Rehydration

From a biochemical and molecular point of view, studying germination is difficult because a population does not complete the process synchronously [45]. Priming treatments are used to synchronize the germination of individual seeds [46]. They initiate germination related processes but prevent emergence of radicle and are followed by drying for storage and marketing. Seed priming causes faster

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germination and better field emergence, which have potential agronomic implications, notably under adverse germination conditions [5]. Optimization of such treatments actually rests on carrying out subsequent germination assays, which only provide retrospective indication of the effectiveness of the priming conditions. Therefore there is strong interest in identifying biochemical and molecular markers of germination and priming for use by the seed industry [47]. A few processes already described to play a significant role during seed priming includes cell cycle related events [48], endosperm weakening by hydrolase activities [49, 50] and mobilization of storage proteins [51].

Germination *sensu stricto* consists of many processes, some can be completed whereas, others may have been started notably during priming. The relationship between endosperm cap weakening (The degrees of β-mannanase activity) and the time of germination after priming has been studied in tomato [6]. Primed and re-dried seeds develop internal free space between the embryo and the endosperm and the most rapidly germinating seeds have the most extensive free space, observed non-destructively using X-ray-radiography [52] and a germination specific β-mannanase gene is expressed in the micropylar endosperm cap region [53]. A strong correlation has been found during osmopriming between lowering of the mechanical restraint, the increase in β-mannanase activity and the appearance of free space which was taken to reflect cell wall hydrolysis [54].

Sung and co-workers [55] found that the endosperm in thermo tolerant cultivars of lettuce had a lower resistance to puncture than thermo sensitive ones and priming reduced the initial force necessary to penetrate the seed and the endosperm in several genotypes. Evidences support that seeds from thermo tolerant lettuce genotypes had higher endo-mannanase activity before radicle protrusion at 35°C than the thermo sensitive ones. Enzyme activities increased during priming of thermo sensitive varieties and therefore might be used as an indicator of priming effects [56]. In dry seeds, for example, tomato and pepper, most embryonic nuclei are in quiescent pre-synthetic GI phase with 2C amounts of DNA. de Castro and co-workers [48] have shown that during imbibition DNA synthesis starts in the radicle meristem tips before cell expansion begins thus entering the G2 phase with 4C DNA. Detailed studies of cell cycle events in tomato pepper and sugar beet using flow cytometry and other techniques have shown that the beneficial effects of priming are associated with the onset of replication and DNA synthetic processes in the radicle meristem nuclei leading to cells stably arrested in the G2 phase after drying. Along with DNA replication occurs the accumulation of β-tubulins which is yet another marker of cell cycle activities in the germinating seeds. Hydration of tomato seeds (Under a range of moisture contents) has shown that β-tubulin accumulation can occur around 29 per cent moisture content, whereas, DNA replication requires a minimum moisture content of around 34 per cent [57].

Extensive studies in tomato seed lots have shown that the water potential and priming temperature greatly alter the priming effects, for example, tomato seeds were subjected to priming at 25°C, and a water potential of –1.5Mpa, a positive linear relationship was found between the frequency of 4C nuclei and the improvement in subsequent germination time. At higher temperature and water potential (>25°C and –2Mpa) germination rates were improved without generating any increase in 4C signals [58]. Also there was no consistent relationship between the frequencies and rates in cauliflower seeds after aerated hydration or osmopriming [59]. Lanteri and co-workers [60] investigated the expression of β-tubulin in the root tips of pepper seeds as a complementary marker for priming. *de novo* synthesis of β-tubulins in response to priming was observed prior to DNA replication after osmopriming for different durations at two water potentials. This observation indicates the possibility of using β-tubulin expression as an additional parameter to differentiate the effectiveness of priming treatments that do not induce nuclear replication.

The oligosaccharides are involved in stabilizing the membranes and protein structure, and in promoting the formation of highly viscous glassy state in the cytoplasm of dry seeds [61].

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However, specific changes in oligosaccharide contents were not associated with the improved longevity of tomato and *impatiens* seeds induced by post priming treatments. Gurusinghe and Bradford [41] found that post priming treatments that substantially restored longevity to hydroprimed tomato seeds only slightly changed sucrose and oligosaccharide content. They observed that an immunoglobulin binding protein termed BiP was induced during partial or slow drying of primed seeds and these are involved in protein repair processes in plants in response to heat shock and other stresses. These could ameliorate stresses incurred during dehydration and storage possibly contributing to improved seed longevity. BiP is a highly conserved member of the heat shock proteins (hsp) 70 family associated with the endoplasmic reticulum [62]. Calcimycin is a calcium ionophore, which causes depletion of Ca_2+ levels of endoplasmic reticulum and is a potent inducer of BiP and related proteins [63, 64]. An increase in BiP mRNA and/or protein abundance was observed in tomato seeds treated with calcimycin after priming. Calcimycin treatments also resulted in an increase in seed longevity equivalent to that induced by heat shock. In addition, it did not induce accumulation of small heat shock protein (Shsps) suggesting that BiP and possibly other ER chaperons rather than a 'mere heat shock response was involved in increasing the longevity of primed seeds. Gallardo and co-workers [65] have identified and characterized specific protein markers that express during priming of *Arabidopsis* seeds. They have detected through 2D electrophoresis three priming associated polypeptides, whose abundance increased during both hydro and osmopriming treatments. A hydropriming specific protein was identified as a catalase isoform. Its abundance increased during hydropriming and continued to increase up to the radicle emergence stage. It is presumed that hydropriming initiates an oxidative stress which generates reactive oxygen species and therefore catalase must be present to minimize cell damage. The abundance of low molecular hsps of 17.4 KD was found to specifically increase in osmoprimed seeds of *Arabidopsis*. These have molecular chaperon activity [66]. These proteins act by maintaining the proper folding of other proteins during the incomplete hydration resulting from soaking of seeds in PEG solution. In the absence of osmotic stress these hsps decline quickly during germination. Thus these specific proteins help as markers to study the priming effect.

Priming Induced Repair Processes in Low Vigour Seeds

Reversal of seed deterioration by priming generally occurs in the meristamatic axis or the radicle tip. Silvritepe and Dourado [67] found that controlled humidification of aged pea seeds to 16-18 per cent prior to sowing decreases chromosomal aberrations, reduces imbibitional injury and improves seed viability. Rao and co-workers [68] reported a reversal of chromosomal damage with partial hydration of lettuce seeds by osmopriming to 33-44 per cent moisture content. Priming also appears to influence germination metabolism in aged axes more than those of non-aged axes. In sweet corn osmo and matriconditioning resulted in decreased conductivity, free sugars and DNA content, whereas, the RNA content increased [69]. Natural aging of french bean seeds, stored up to 4 years, showed membrane disruption and leakage of UV-absorbing substances which was ameliorated by hydropriming [70, 71]. Lower electrical conductivity following hydropriming indicated reduced membrane leakage for egg plant and radish [72] and onion [73] seeds. These beneficial effects may be due to the flushing of solutes from the seeds during the priming procedure. As a result primed seeds often perform better in disease-infested soils because of decreased electrolyte leakage and faster germination which reduce the window of opportunity for fungal attack [74].

Priming has been observed to increase the activity of several enzymes and counteract the effect of lipid peroxidation. Saha and co-workers [75] showed that matricpriming caused increased amylase and dehydrogenase activity in aged soyabean axes compared to untreated (Control) seeds. In wheat, osmopriming increased protein and DNA synthesis [76]. L-isoaspartyl methyl esterase enzymes initiate the conversion of detrimental L-isoaspartyl residues to normal L-isoaspartyl residues that accumulate in

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naturally aged wheat seeds [77]. This enzyme is present in 45 species from 23 families representing most of the divisions of the plant kingdom. Osmoprimed tomato seeds subjected to accelerated aging showed restored activity of this enzyme to levels similar to non-aged controls. Kester and co-workers [78] suggested that this enzyme is involved in the early repair of deteriorated seeds. Also osmopriming has been reported to resume the loss of peroxide detoxifying enzymes *viz.*, catalase, superoxide dismutase and glutothione reductase in aged sunflower seeds by raising the levels of these to the same extent as non-aged seeds [79]. Chiu and co-workers [80] found that increasing hydration enhanced membrane repair in watermelon seeds and attributed to the stimulation of peroxide scavenging enzymes that controlled ageing by counteracting lipid peroxidation.

Changes in nucleic acid levels following priming have been reported. ATP levels increased in kohlrabi, spinach, eggplant and pepper seeds during osmopriming [81]. Osmopriming also increased the RNA content of leek [82]. Powell and co-workers [59] observed that both aerated hydration and osmopriming of high vigour *Brassica* seeds resulted in increased proportions of 4C DNA and β-tubulin along with germination advancement. In contrast there was no change in the 4C DNA in low vigour seeds although the β-tubulin content increased suggesting that germination advancement has begun but was delayed as compared to high vigour seeds. These seeds performed better during controlled deterioration test by maintaining better germination than those of high vigour seeds. This is explained by the fact that cells in the G2 phase of cell division containing 4C DNA are more sensitive to factors affecting nuclear division and chromosome morphology. Thus the high vigour seeds showing increased 4C DNA were more sensitive to both drying after seed priming treatment and a period of storage; and hence reduced vigour and viability. In contrast, the absence of or low 4C DNA in low vigour seeds may result, from their capacity, to undergo metabolic repair during initial stages of hydration.

FUTURE NEEDS

Developing a complete priming protocol must address the balance between germination advancement and reduced seed longevity. Attempt to attain the most rapid germination from priming must be weighed against the enhanced liability of this pre-sowing treatment. Post treatment drying and shelf-life problems should be tackled to ensure consistent performance over a wider range of storage conditions. The challenges of technology to enhance seed performance, provides an opportunity for more in-depth studies on physiological and biochemical changes that occur during seed treatments. Physiological, biochemical and biophysical markers are needed to detect the enhanced quality of seeds.

Molecular tools are dramatically enhancing our knowledge of the biochemical and regulatory pathways underlying the complex physiological and developmental process of germination. Genomic and transgenic approaches can establish the timings and identity the specially activated genes and their tissue expression patterns or the consequences of specific gene inactivation and provide insights into their functions. Proteomics and C DNA micro-array technology may prove valuable tools providing simultaneous information over a multitude of processes. One of the many stimulating outcomes from using the potential wealth of this type of information in classical physiological approaches could be identification of diagnostic markers, which might be employed to interrogate gene expression to characterize seed quality and to develop and optimize priming enhancement procedures. These markers would have practical application in quality control programmes as tools to develop and monitor hydration events and possibility for upgrading seed quality.

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