Pre-sowing treatments to improve seed performance

P. Coolbear

Seed Technology Centre, Department of Plant Science, Massey University, Palmerston North, New Zealand.

Abstract

Physiological pre-sowing treatments designed to improve germination performance are becoming increasingly fashionable, especially for vegetable seeds. Advantages in rates and uniformity of germination and, occasionally, stress avoidance have been shown to result from such treatments in certain situations.

Most of these techniques involve some form of controlled pre-imbibition of the seed which allows the initiation of early germination processes. This is usually followed by drying to near original moisture contents so that seed can be handled by conventional machinery. Citing examples from recent and current work undertaken in the U.K. and the Seed Technology Centre, this paper examines aspects of the physiology of the process and some of the potential of these treatments for farmers and growers in New Zealand. Current problems, notably the reproducibility of responses to treatment by different seedlots and problems associated with the storage of treated seed are also discussed.

Additional key words: *germination rate, seed priming, seed storage, seedling establishment, uniformity.*

Introduction

The aim of this paper is to review some of the current ideas and recent work on physiological pre-sowing treatments designed to enhance seed germination performance. These treatments (referred to by a multitude of names, such as germination advancement, osmoconditioning, invigoration, hardening or, more usually in current literature, priming) are designed to give the seed a 'head start' by facilitating a controlled initiation of some of the physiological processes of germination prior to sowing. As such they are distinct from chemical seed treatments (e.g., applications of pesticides or fertilizers to seeds) or physical seed treatments (such as the scarification of hard seeds prior to sowing).

Over the past thirty years there has been extensive literature published on seed priming and related treatments. These have been particularly promising for high value small seeded vegetables where rapid, uniform germination is at a premium either in the cell transplant situation or where accurate and uniform plant population density is required from direct drilling. Primed seeds of carrot, cauliflower, endive, lettuce, leek, onion, peper and tomato are now marketed commercially, especially from the U.S. and Europe. It should be appreciated that this kind of treatment is usually just one component of an integrated quality improvement and enhancement package where particular attention has also been paid to cultivar selection, providing optimal conditions during seed production, careful processing, grading, packaging and storage. The primed seed may be further enhanced by a high-technology seed coat.

Methodologies of Priming Treatments

The basic idea of physiological pre-sowing treatments is to allow the seed to take up sufficient water to initiate early events in germination, but not to allow radicle emergence. Seeds can be subsequently dried down to near original moisture content and then handled conventionally. The different methods of controlling seed hydration during priming are:

- Wetting - drying cycles
- Imbibition at low temperatures (low temperature pre-sowing treatments, LTPST)
- Use of inert osmotic solute (e.g., polyethylene glycol, PEG).
- Use of salt solutions (e.g., KNO_3 + KH_2 PO_4)
- Use of silicates or gels (matric priming)
- Controlled addition of water (drum priming)

A constant problem of wetting-drying treatments has been difficulties in controlling the process. There is always a danger that germinative metabolism might be allowed to proceed too far and seeds would then lose their desiccation tolerance and be damaged by the subsequent drying back necessary to enable them to be handled like conventional seed (this usually occurs at visible radicle emergence, e.g., Berrie and Drennan, 1971). Accordingly, various approaches have been developed to slow the process down and make it more controllable. One of these has been the low temperature pre-sowing treatment method which we have extensively investigated for tomato seeds (e.g., Coolbear *et al.*, 1984; Coolbear and McGill, 1990) and involves holding the seeds imbibed in distilled water at 10°C for several days before drying-back. While this method has met with some success, the approach relies on the relatively high minimum temperature for germination of this species (8-10°C) and is much less appropriate for species with lower germination minima. Accordingly, osmotic or priming treatments have been preferred. These utilise either salt solutions or an inert, water-soluble osmoticum such as polyethylene glycol (PEG) as their substrate.

There is considerable debate about which of these two treatment methods is the most appropriate for different seeds. PEG 8000 (or 6000) is a large molecule which does not penetrate the seed. This means that the osmotic environment can be regulated quite precisely, but, on the other hand, aqueous solutions of PEG are viscous, difficult to aerate adequately and generally not easy to handle. They are also liable to become infected with microflora (Petch *et al.*, 1991). Commonly used salts are KNO_3 and KH_2PO_4. Both may enter the seed during treatment and either this or more efficient aeration seems to provide additional advantages over PEG for some species, e.g., tomato (Haigh and Barlow, 1987a; Alvarado *et al.*, 1987) and pepper (Rivas *et al.*, 1984). In other cases penetration of ions into the seed may have deleterious effects and PEG is a more effective treatment medium, e.g., in beet (Khan *et a*l., 1983) and carrots and celery (Brocklehurst and Dearman, 1984).

Often in the laboratory such treatments are achieved by allowing seeds to imbibe in Petri dishes, but on a commercial scale bulk treatment methods must be developed, aerated columns being the most favoured approach. Recently, however, two more manageable approaches have been developed. One of these, solid matrix priming or 'matriconditioning' (Taylor *et al.*, 1988; Khan *et al.*, 1990) utilises substrates such as vermiculite and synthetic calcium silicates which have high water retention and thus generate a matrix potential rather than an osmotic potential. Seeds are mixed with water (or treatment solution) and substrate, usually in a substrate to seed ratio of 0.4 or less. The fine substrate adheres to the seed coat and not only controls water uptake by the seed during the conditioning period, but has the potential to act as a carrier for other chemicals and/or beneficial micro-organisms which might be used as supplementary treatments. As yet this approach needs further evaluation both in the laboratory and in the field, but results are promising, not just with the small seeded vegetables, but also with maize (Parera and Cantliffe, 1991). Success has also been obtained with larger legume seeds (Khan *et al.*, 1990): because of their susceptibility to soaking injury, these seeds are not easy to prime by more conventional methods.

An alternative, which is possibly easier to apply on a commercial scale, is to regulate the amount of water to be added to the seeds by mechanical means. This is drum priming, patented by Hugh Rowse at Wellesbourne in the U.K. (Rowse, 1987; Gray *et al.*, 1990). In this method seeds are placed in a rotating drum and water added in a carefully regulated way, while at the same time ensuring thorough and continuous mixing of the seed bulk. This development of the priming technique has already been adopted commercially and is predicted to have a most promising future for vegetable seeds.

Evaluation of Pre-sowing Treatments

Table 1 summarises the potential effects of priming treatments with a brief assessment of their level of success. Various issues in this Table will be discussed in this section using examples from the literature and, more particularly, our own research on tomato seeds.

Effects on seed germination

Figure 1 shows a typical set of results of a trial to determine the optimal low temperature pre-sowing treatment (LTPST) duration for tomato seeds, cv. Grosse Lisse. LTPST for 14 d allows maximal reduction in time to 50% radicle emergence (T50) and greatest reduction in the spread of germination (T90-T10), without major loss of seed by germination during the pre-sowing treatment. We have achieved similar effects by priming tomato seeds in polyethylene glycol (PEG), but comparative studies indicate that the LTPST method gives improved uniformity of radicle emergence (Coolbear *et al.*, 1987).

The germination data in Figure 1 were obtained from radicle emergence trials conducted at 20°C and result in a reduction in median germination time approaching 50%. Extensive studies by our group on tomato seeds have shown that improvements in seed germination rates are a simple function of the T50 of untreated seeds, either between cultivars and seedlots (Ranganarasimhiah, 1989) or within a single lot under a range of germination conditions (Coolbear and McGill, 1990). In contrast, improvements in uniformity of germination are highly variable. Early ideas on osmotic pre-sowing treatments were that imposition of an osmotic constraint would hold the more vigorous seeds of a population at the brink of visible germination without any deleterious effects, while allowing less vigorous ones to catch up, thus considerably improving the uniformity of the seedlot (Heydecker and Gibbins, 1978). Recent work seems to indicate that this is not the case. Both Brocklehurst and Dearman (1983) and ourselves (Efendi, 1991) have found in onion that while the ability of osmotic pre-sowing treatments to improve the rate of germination is highly reproducible and predictable, treatment effects on uniformity vary considerably with seedlot. Similarly, in a comparative study of the responses to LTPST by seeds of a total of ten different seedlots from three cultivars commercially available in New Zealand, by no means all seedlots showed a clear improvement in uniformity of radicle emergence (Fig. 2).

Table 1. The different objectives of physiological pre-sowing (priming) treatments and a brief assessment of the level to which they have been achieved.

Increase the rate of seed germination and emergence:
predictable and reproducible success for small seeded vegetables;
some successes with other crops.

Increase the uniformity of germination and emergence:
results vary tremendously with seed lot.

Advance seedling growth:
occurs as a result of advancement of radicle emergence;
little or no evidence for increased seedling relativegrowth rates.

Modify seedling growth:
not usually; possibly with added PGR's.

Advance yield:
successes with several crops; a large component likely to be stress avoidance at key times during crop growth.

Stress avoidance:
spectacular success in some situations.

Allow the repair of deteriorated seeds:
demonstrated in the laboratory.

Prolong the storage life of seeds:
occasional positive results reported;
in general, treated seeds deteriorate rapidly under adverse storage conditions.
results vary considerably with seed lots.

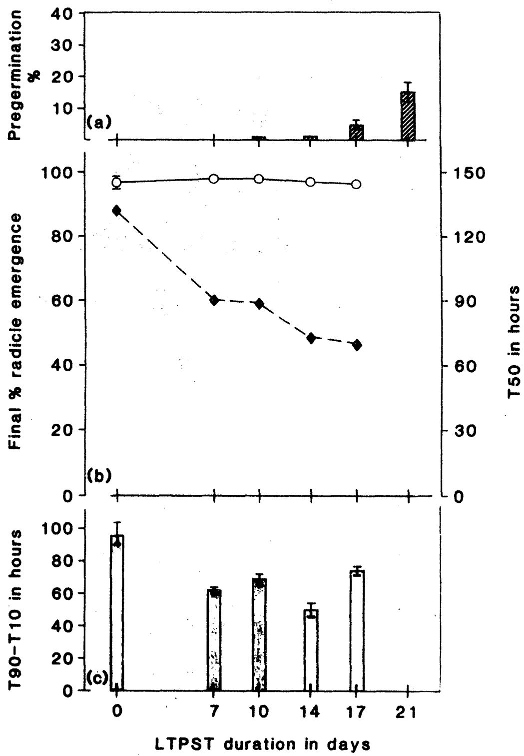

Figure 1. Effects of different durations of low temperature pre-sowing treatment (LTPST) on the germination behaviour of tomato seeds, cv. Grosse Lisse. (a) Germination during pre-sowing treatment; (b) final radicle emergence percentage (O) and time to fifty-percent radicle emergence. T50 (♦) ; (c) uniformity of germination as the time interval between ninety percent and ten percent radicle emergence. Data presented are the means of four replicate germination trials. Individual standard errors are shown where larger than the symbols used. (Data from Ranganarasimhiah, 1989).

Effects on seedling growth and plant establishment

Another controversial area of pre-sowing treatments has been the question of whether or not they modify seedling growth *per se*. Reported benefits of treatment on vegetative growth or yield are usually attributable to the effects of earlier emergence (and possible stress avoidance: see later) rather than any treatment-induced improvement of relative growth rates. In the mid 1980's we undertook a careful evaluation of the effects of seed treatment on early seedling growth of tomatoes (Coolbear *et al.*, 1987). In Petri dish trials there was evidence that axis growth of seedlings from untreated seeds may be slightly greater than treated ones (Fig. 3), however, in emergence trials from soil, shoot growth rates of autotrophic seedlings were essentially identical.

In the early 1960's (e.g., May *et al.*, 1962 and review by Heydecker and Coolbear, 1977), much attention was focused on hydration-dehydration type treatments of seeds with some spectacular results being reported from Russia. For example Mart'janova *et al.* (1961) recorded a 105% increase in yield in tomatoes as a result of one cycle of wetting and drying of the seed before sowing. It is now clear that these treatment benefits are largely

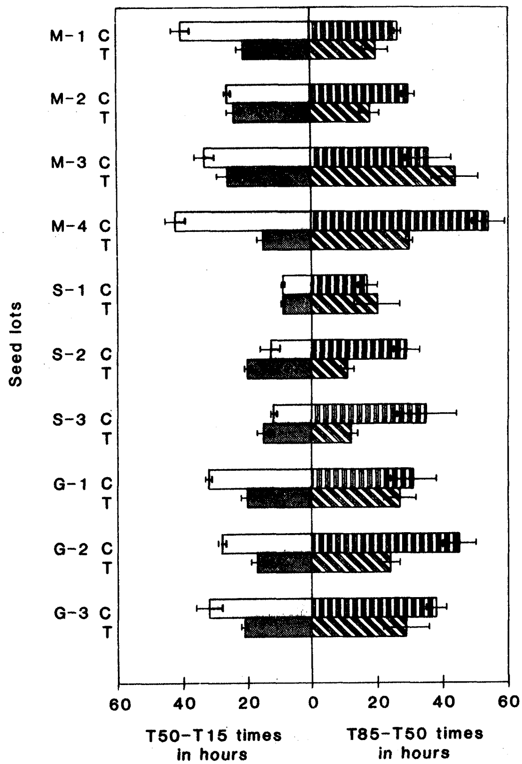

Figure 2. Uniformity of radicle emergence of LTPST - treated seeds (T) or untreated controls (C) of ten different seedlots of tomato, cv's Moneymaker (M), Scoresby Dwarf (S) and Grosse Lisse (G). Uniformity was measured as two components: (a) as the time intervals between 15 and 50% germination and (b) the intervals between 50 and 85% germination. Data are means of four replicate germination tests, SE's are shown. (Data from Ranganarasimhiah, 1989).

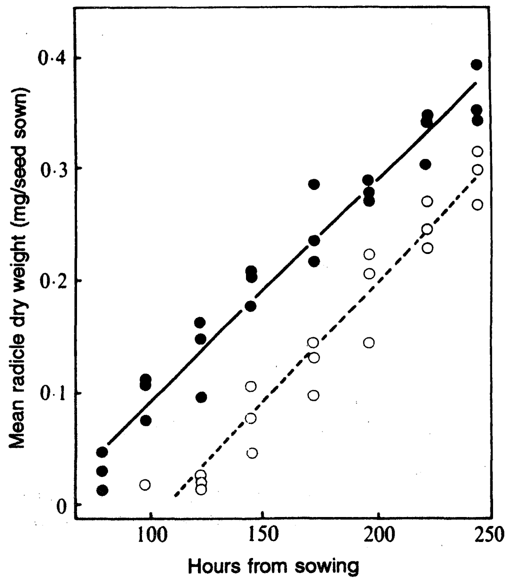

Figure 3. Initial stages of radicle growth of tomato seeds, cv. Moneymaker with (●) or without (O) LTPST. Calculated rates of radicle growth are 1.98 µg dry matter $seed^{-1}$ h^{-1} for treated seeds and 2.15 µg $seed^{-1}$ h^{-1} for untreated seeds ($P<0.001$). Data reproduced with permission from Coolbear *et al.* (1987).

those of drought avoidance rather than drought tolerance, in that seeds are induced to germinate faster and seedlings thus become established much more quickly. The most spectacular example of this type of stress avoidance strategy is work by Cantliffe's group using salt treatments to improve the germination of lettuce seeds which are allowed to progress through the stage at which they are liable to thermodormancy during the pretreatment process. Treated seeds can then successfully germinate in the southern U.S. when summer temperatures in dark soils may exceed 40°C, conditions more than sufficient to throw untreated seeds into secondary dormancy (Perkins-Veazie and Cantliffe, 1984). Figure 4 shows some corresponding results from our laboratory with LTPST treated tomato seeds. Presowing treatment allows significantly higher final radicle emergence under a combination of elevated temperature and water stress.

Seed priming and seed deterioration

The final area of practical concern for seed priming is the interaction between pre-sowing treatments and seed deterioration. It has been demonstrated by several workers (e.g., Coolbear *et al.*, 1984; Dearman *et al.*, 1986) that priming treatments have the ability to repair partially deteriorated but still viable seed. It seems that the controlled water uptake and extended period of pre-germinative hydration in a typical pre-sowing treatment facilitate the operation of the seeds' inbuilt repair and detoxification systems (e.g., see Priestley, 1986). Whilst in general it is clear that the best commercial potential for priming is the enhancement of performance of seeds of the highest quality (e.g., Perkins-Veazie and Cantliffe, 1984), such repair treatments may be appropriate in the cases of valuable deteriorated stock or rare genetic material.

The other side of this equation is the storability of seed after treatment. It is now generally accepted (e.g., Odell and Cantliffe, 1986; Dearman *et al.*, 1987; Alvarado and Bradford, 1988) that pre-treated seed need to be stored with care and, at least under high moisture conditions, deteriorate more rapidly than untreated seeds (e.g., Figure 5 which shows some work from our laboratory for treated tomato seeds). This observation raises some interesting issues concerning relationships between seed vigour and viability. It has often been argued that seed deterioration is a simple and cumulative sequence of events, in that processes leading to loss of seed viability are simply a continuation of those leading to loss of vigour (e.g., Ellis and Roberts, 1981; Dearman *et al.*, 1986), but data like those in Figure 5 suggest that while some aspects of vigour, i.e., germination rates, can be enhanced, other aspects, i.e., storability, are actually impaired.

Mechanisms of Pre-sowing Treatments

The mechanisms by which pre-sowing treatments enhance seed performance are not fully understood, but must affect both the general maintenance processes of germinative metabolism which survive drying back and key rate-limiting steps. Coolbear *et al.* (1990)

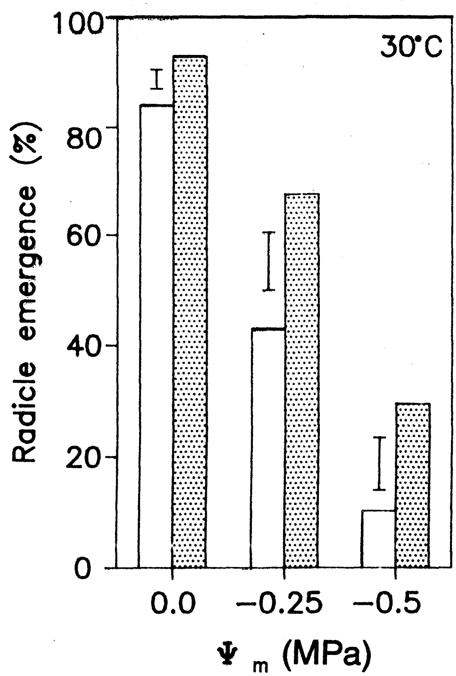

Figure 4. The effect of LTPST on radicle emergence of tomato seeds cv. Moneymaker under temperature and osmotic stress. Treated (shaded histograms) and untreated (open histograms) seed were germinated at 30°C in solutions of polyethylene glycol 6000 at the osmotic potentials indicated. Data are means of five replications and the $LSD_{0.05}$ between each pair of means are shown. Reproduced with permission from Coolbear and McGill (1990).

demonstrated that in tomatoes subject to LTPST there was considerable ribonucleic acid synthesis which was involved in replacement of damaged ribosomes. We also showed, however, that this was not a rate limiting process. Increased respiratory activity has been reportedly associated with pre-sowing treatments, e.g., Koehler (1967); Malnassy (1971) and work with *Pinus radiata* in this laboratory (Kusmintardjo, unpublished). It is difficult, however, to determine whether enhanced oxygen uptake rates are a cause or a consequence of more rapid germination.

Much attention has recently been focused on three possible rate-limiting steps in the germination process and whether or not priming affects these directly. Given that visible germination is due to radicle elongation, these are:

a) osmotic adjustment
b) weakening of tissues surrounding the embryo and,
c) the synthetic capacity for embryo cell expansion.

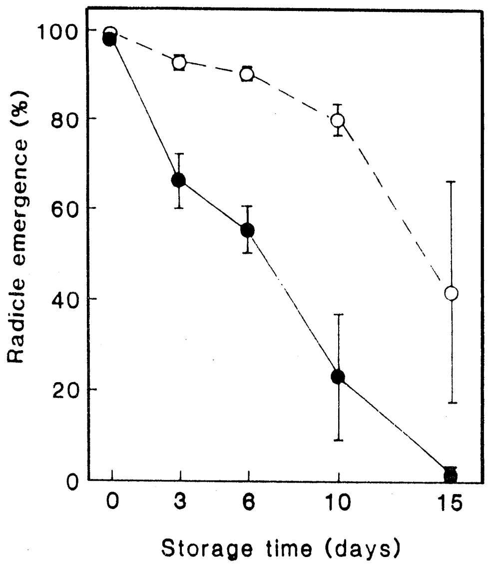

Figure 5. Storability of LTPST treated (●) and untreated (O) tomato seeds, cv. Grosse Lisse at 40°C, 40% SMC. Data are means of four replications with individual SE's shown. (From Ranganarasimhiah, 1989).

Bradford (1986) produced a strong argument for osmotic adjustment being the key rate limiting process advanced by pre-sowing treatments of lettuce seeds. Increased mobilisation of solutes during priming allows a reduction in cell osmotic potential and thus greater water uptake to drive cell expansion. Certainly we have found that treated tomato seeds have a greater water uptake capacity then untreated seeds (Table 2), although we have yet to find any evidence of increased solute concentration within the embryo (Coolbear and McGill, unpublished). This agrees with the findings of Haigh and Barlow (1987b) who showed that the osmotic potential of tomato seed embryos in the lag phase of imbibition was around -1.5 MPa and any osmotic adjustment was thus unnecessary. They suggest that the main limitations in germination in tomato seeds is the physical constraint of the surrounding endosperm tissue which must be digested before radicle emergence. Karssen *et al.* (1989) showed that one of the functions of seed priming was to increase the extra-embryo enzyme activity responsible for weakening the endosperm. An alternative suggestion as a rate-limiting step for tomatoes under low temperature conditions was put forward by Liptay and Schopfer (1983) who suggested that the ability of embryo cells to initiate the metabolism necessary for extension growth was of prime importance.

Table 2. Moisture contents (% dwt basis) of treated and untreated seeds in early lag phase of imbibition (28 - 32 h).

	Osmotic potential of imbibition medium (MPa)	
	0.0	-0.5
Treated	87.8	92.4
Untreated	82.3	82.1

LSD $_{0.05}$ = 2.58

— Coolbear and McGill (unpublished data)

What emerges from this debate is the likelihood that pre-sowing treatments may have performance enhancing effects via a range of rate-limiting and non-rate limiting steps. The relative importance of these effects may vary not only between species but even amongst seedlots.

Conclusions

From the foregoing discussion, it can be seen that physiological pre-sowing treatments do have their limitations. They are not the solution to all agronomic/horticultural establishment problems nor can they provide a substitute for poor initial seed quality. Nevertheless, they do have considerable potential for some applications, especially those requiring rapid, predictable emergence. As commercial development of these techniques proceeds we need to gain a clearer understanding of what priming can be expected to do for New Zealand growers and how best to apply the technology.

A key difficulty in commercial practice is that, for outdoor planting at least, priming treatments are largely insurance. When planting conditions are optimal the benefits of physiological pre-sowing treatments over the use of good quality untreated seed are likely to be small. Under marginal conditions, however, the advantage are likely to be dramatic. On this basis it is likely that development of appropriate priming strategies for commercial practice in New Zealand will require several years of accumulated experience. One key decision is whether priming can be used simply as a one-off 'add-on' technology to commercially available seeds or whether it is better employed as an integral component of a quality enhancement package as is currently marketed by some U.S. and European seed firms. Answers to this kind of question are likely to be market-driven rather than purely scientific and are equally likely to vary with individual circumstances. Alongside this kind of developmental work, there is a clear need for a greater understanding of the mechanisms by which these seed treatments act. In view of the variation between seedlots, a major focus of future research should be towards an increased understanding of the interactions between seed production and processing conditions with subsequent priming effects.

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