Induction of longevity in primed seeds

G.T. Bruggink*, J.J.J. Ooms and P. van der Toorn
Novartis Seeds, PO Box 2, 1600 AA Enkhuizen, The Netherlands

Abstract

Priming of seeds is generally intended to reduce time to germination, often leading to improved emergence. However, as a negative side effect, priming reduces longevity of seeds. We studied the possibilities to obtain primed seeds with reduced time to germination but with longevity similar to that of untreated seeds. For several species tested we found that the desired longevity could be obtained by keeping the seeds, after a priming treatment, under a mild water and / or temperature stress for a period of several hours to days. Time to germination did not increase again due to such a treatment. Optimal duration and degree of water stress were strongly temperature dependent. The methods applied to obtain primed seeds without loss of longevity are very similar to those used to induce desiccation tolerance in germinated seeds.

Keywords: Priming, longevity, desiccation tolerance

Introduction

Seeds of orthodox plant species are able to survive dry storage for long periods of time. To survive dry storage, freshly harvested seeds must first survive desiccation. Ooms *et al.* (1993) have shown that desiccation tolerance should be expressed as the period that a seed survives dry storage. It was also shown by Ellis and Hong (1994) and Hay and Probert (1995) that seed longevity and desiccation tolerance are closely related. Therefore, the mechanisms that play a role in desiccation tolerance may be equally important in relation to dry storage. Leprince *et al.* (1993) concluded that desiccation tolerance is a multifactorial property in which different components are important. Sucrose and oligosaccharide content (Leprince *et al*., 1990a; Blackman *et al*., 1992; Bernal-Lugo *et al*., 1993; Blackman and Leopold, 1993; Ooms *et al*., 1993) and certain heat-soluble proteins referred to as Late Embryogenesis Abundant (LEA) proteins (Blackman *et al*., 1991) are thought to play key roles. Also, protection against free radicals produced during dehydration could play a role in desiccation tolerance. Free radicals would lead to damage to membranes in the absence of protection mechanisms (Sargent *et al*., 1981; Senaratna and McKersie, 1983; Leprince *et al*., 1990b).

Vegetable and flower seeds can be primed to reduce the time to germination, often leading to improved emergence, especially under adverse conditions. This treatment is based on the addition of water to the seed in a controlled environment (Heydecker *et al*., 1973). An important practical limitation of seed priming is the strong reduction in longevity that is associated with the desired increase in speed of germination. This was shown, for example, for lettuce (Tarquis and Bradford, 1992) and pepper (Saracco *et al*., 1995). For practical reasons longevity is often quantified by the survival of seeds in a controlled deterioration test, in which longevity is strongly reduced by exposing seeds to high temperature, usually in combination with a high relative humidity (RH) (Powell and Matthews, 1984a, b).

We have described treatments that induce desiccation tolerance in germinated seeds (Bruggink and van der Toorn, 1995). Assuming that mechanisms conferring longevity and desiccation tolerance are related, treatments that induce desiccation tolerance should also have a positive effect on longevity. In this paper we have tested this hypothesis by subjecting primed seed to such induction treatments.

Material and methods

Imbibition treatments

Impatiens seeds (*Impatiens walleriana* Hook. f. 'Impulse red', Novartis Seeds B.V.) (100 g) were imbibed in 1 l of aerated water for 3 d at a temperature of 20°C. Seeds were then centrifuge dried and subsequently either desiccated or incubated before desiccation.

Priming treatments

Priming of pansy (*Viola* × *wittrockiana*, different colours from the 'Roc' series, Novartis Seeds B.V.) and pepper seeds (*Capsicum annuum* L., cv. Bell Tower,

*Correspondence
Email: tonko.bruggink@seeds.novartis.com
Fax: +31 228 312818

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Novartis Seeds B.V.) was carried out by imbibing the seeds until they reached the desired moisture content of about 38% (fresh weight basis), after which they were transferred to a rotating drum. Seeds were kept in the drum for 3–4 d at a temperature of 20°C. At the end of a priming treatment seeds were either dried in one of the ways described below, or they received a further incubation treatment.

Desiccation

At the end of imbibition, priming or incubation treatments, seeds were desiccated rapidly by means of exposing them to moving air of 20°C and 40% RH. This led to a decrease in moisture content of 5–10% per hour. This desiccation method is referred to as 'fast drying'. Alternatively, seeds were dried slowly by exposing them to standing air of 20°C and 75% RH for 3 d. This led to a decrease in moisture content of 0.1–0.3% per hour, referred to as 'slow drying'. At the end of such a treatment, seeds were transferred to fast drying conditions to ensure complete desiccation.

Incubation

After imbibition or priming, different methods of incubation were applied:

1. Imbibed seeds were transferred to 1 l of aerated PEG solution (340 g PEG 8000 l^{-1}). After a specified number of days in this solution seeds were rinsed, centrifuged and desiccated by means of fast drying.
2. Primed seeds were dried (fast-drying method for a short period of time) until they had reached a specified moisture content and then kept in 180 ml plastic containers (Greiner) at a specified temperature for a specified period of time. It is assumed that RH was in equilibrium with the moisture content of the seeds. Seeds were then desiccated by means of fast drying.
3. A heat shock was applied to primed seeds after bringing 5 g of seeds to a certain moisture content and sealing them in aluminium bags. These were then kept in a waterbath at 40°C for 3 h and subsequently desiccated by means of fast drying.

Moisture determination

Seed moisture content was determined by weighing a 1 g sample of seeds before and after oven-drying at 130°C for 2 h. Moisture content is expressed as a percentage of fresh weight.

Germination, t_{50} and controlled deterioration tests

Germination of seeds was tested by placing 2 samples of 100 seeds on moist blotter paper at 20°C. Final germination percentages were scored after 2 weeks. For t_{50} determinations 2 samples of 100 seeds were sown in the same way. From daily counts of radicle emergence the t_{50} was calculated (Orchard, 1977). For controlled deterioration (CD) tests 1–2 g of seeds were equilibrated at RH = 40% (Impatiens) or RH = 75% (pepper and pansy), and sealed in aluminium bags which were placed in a water bath of 50°C for a specified period of time. Seeds were then germinated as mentioned above, and their germination percentage was used as a measure for their ability to withstand deterioration.

Results

Initially we studied whether the methods described earlier to induce desiccation tolerance in germinated seeds would have an effect on longevity of ungerminated seeds. Impatiens seeds were imbibed in water at 20°C for 3 d at which time no germination was visible (time to first germination was 3.5–4 d for this seedlot). After 3 d imbibition, seeds still survived desiccation but had largely lost the ability to survive in a CD test (Table 1). Incubation of seeds in PEG solution, after imbibition but prior to desiccation, reinstated the ability to survive in a CD test (Table 1). The percentage of seeds surviving after the CD test was similar to that for control seeds. An incubation for 3 d was sufficient to restore longevity.

Subsequently, the effect of a modified treatment on primed pansy seeds was studied. Seeds of 4 lots were primed (3 d at 20°C). At the end of the priming treatment the batches were split in two. One half was exposed to fast drying conditions, the other to slow drying conditions for 3 d, prior to final fast drying. Samples of seeds were then subjected to a t_{50} test and a CD test, and the remainder stored at 18°C and 30% RH for 23 months, after which they were germinated. Immediately after treatment there was no difference in

Table 1. Effect of incubation in PEG 8000 (324 g l^{-1}; 8°C) on the ability of Impatiens seeds to withstand controlled deterioration. Before incubation seeds had been brought close to germination by an imbibition in water at 20°C for 3 d. After the incubation seeds were rinsed and exposed to fast drying conditions followed by a CD test. CD 0, 48 and 96 is percentage germination after 0, 48 and 96 h of controlled deterioration, respectively

Treatment	CD 0 (%)	CD 48 (%)	CD 96 (%)
control (non-incubated)	93	79	69
3 d water only	86	19	2
3 d water + 1 d PEG	94	68	18
3 d water + 3 d PEG	93	92	75
3 d water + 7 d PEG	96	84	65

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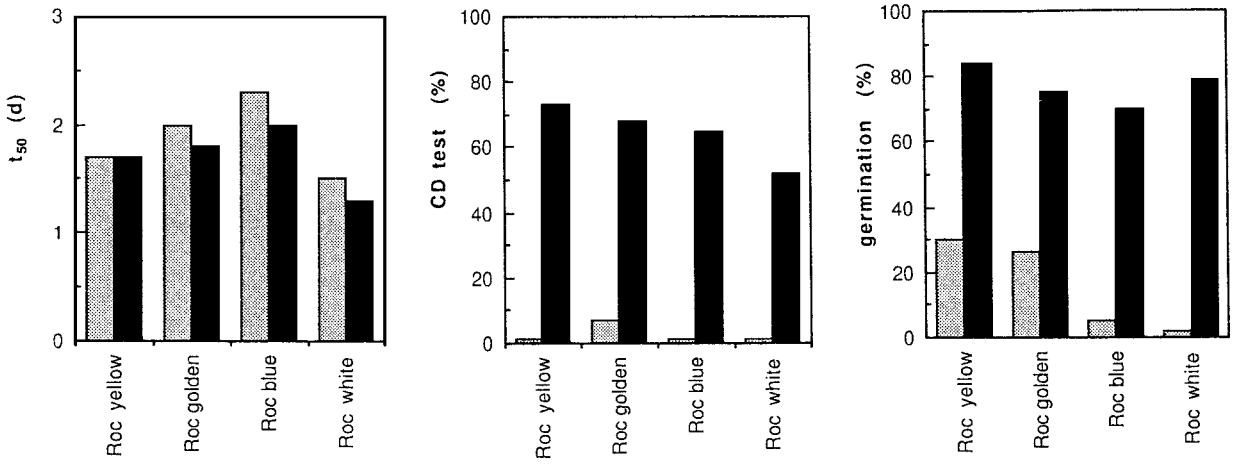

Figure 1. Effect of drying method on t_{50} and longevity of primed pansy seeds. Left: t_{50} of pansy seeds of 4 varieties which were either dried slowly (black) or fast (grey) after priming. Middle: survival of these seeds after 24 h of controlled deterioration. Right: germination of these seeds after 23 months of storage.

final germination between the two treatments (not shown). Figure 1 shows that the two treatments also resulted in similar values for t_{50}. However, the slowly dried seeds deteriorated considerably less during a CD test and also showed much better germination after storage for 23 months than the seeds which experienced fast drying after priming.

The role of temperature was studied in a following experiment. Pepper seeds were primed for 4 d in a rotating drum and either desiccated immediately or incubated under different conditions before final drying. These conditions consisted of incubation in a closed container for 2 d at a seed moisture content which was either the same as during priming or lowered by 5%, and at a temperature of either 20 or 35°C. Notice that no treatment with priming moisture content and 20°C was present since this would be a prolonged priming treatment, leading to further loss of longevity. As in the previous experiments, survival after CD was low for seeds that were directly desiccated after priming but improved considerably when desiccation was preceded by an induction treatment (Table 2). The improvement in longevity was most obvious after an incubation at high temperature in combination with a lowered moisture content, while t_{50} changed hardly if at all due to the incubation treatments.

Table 2. Effect of longevity induction treatments on t_{50} and CD test survival of primed pepper seeds. Before final desiccation seeds were exposed to the mentioned treatments. CD 48 indicates the germination percentage after 48 h of controlled deterioration

Treatment	t_{50} (d)	CD 48 (%)
control (non-treated)	4.6	37
primed only	1.6	0
primed + 2 d 20°C at lowered MC	1.6	27
primed + 2 d 35°C at priming MC	1.5	44
primed + 2 d 35°C at lowered MC	1.6	74

In order to study the interaction between temperature and moisture content further, primed pepper seeds were either exposed to fast-drying conditions for immediate desiccation or their moisture content was lowered to varying extents. Subsequently they were stored, essentially without further moisture loss, for periods of 1 d at 32°C, for 2 d at 20°C or for 3 d at 8°C, before final desiccation. Time to 50% germination, which was 5 d for untreated seeds (not shown) and 1.8 d for primed, directly desiccated seeds, was further reduced by most incubation treatments, especially those at high moisture contents (Fig. 2). The exception to this was treatments at low moisture content at 32°C, where t_{50} increased.

The CD test result, which was 80% survival for untreated seeds (not shown) and 30% survival for primed, directly desiccated seeds, was hardly influenced by incubations at 8°C, except for incubation at 38% moisture content where survival decreased (Fig. 2). Incubation at 20°C was effective in improving the survival at moisture contents between 20 and 35%, but had a negative effect at a moisture content of 38%. Incubation at 32°C was effective over the range of moisture contents of 20–38% in improving the CD test result, although at lower moisture contents there was an increase in t_{50} after the incubation treatment.

In addition, the effect of a heat shock on longevity of primed pepper seeds was studied. Pepper seeds were either directly dried after priming or they were dried to varying degrees and, after sealing in

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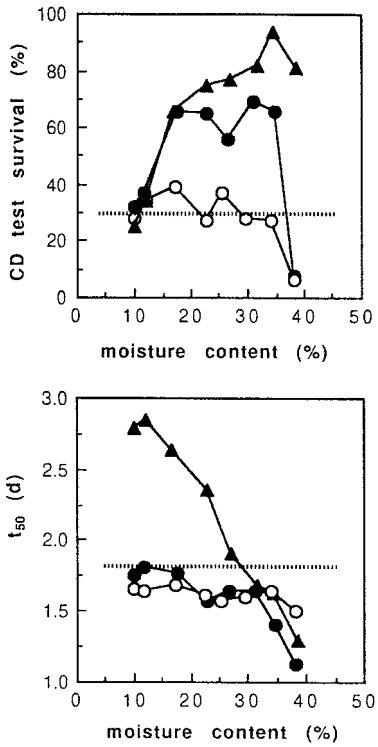

Figure 2. Effect of induction treatments at different temperatures and seed moisture contents on t_{50} and survival in a CD test of primed pepper seeds. Horizontal dotted lines in the figures indicate the values for non-induced seeds (i.e. directly desiccated after priming). Moisture content at the end of priming was 38%, incubation took place at moisture contents between 10 and 38% at temperatures of 8°C (open circles), 20°C (closed circles) or 32°C (triangles), for 3, 2 and 1 d, respectively.

aluminium bags, exposed to a temperature of 40°C for 3 h, before final desiccation. Table 3 shows that survival in a CD test strongly increased after the heat shock treatment. Best results were obtained at the highest moisture content.

Discussion

In comparison to untreated seeds, primed seeds were characterized by a reduction in t_{50} and a parallel reduction in survival after a CD test (Tables 1, 2 and 3). Figure 1 illustrates that the reduced longevity of primed pansy seeds after storage correlated with a low survival in the CD test. This confirms the assumption that reduced survival in CD tests is predictive for decreased longevity under commercial storage conditions.

A number of treatments, applied after the priming treatment, were able to prevent the reduction in longevity. Such methods consisted of PEG incubation

Table 3. Effect of a heat shock of 40°C for 3 h at different moisture contents (MC), prior to desiccation, on CD test survival of primed pepper seeds

Treatment	CD 48 (%)
control (non-treated)	75
primed only	14
primed + heat shock at 35.3% MC	62
primed + heat shock at 30.6% MC	46
primed + heat shock at 20.0% MC	44
primed + heat shock at 14.8% MC	46
primed + heat shock at 9.6% MC	24

of seeds on the brink of germination (Table 1), slow drying of primed seeds by means of exposure to air of 75% RH (Fig. 1) and incubation in high RH of primed seeds, which were brought to a certain moisture content (Fig. 2, Table 2). Also a heat shock treatment of 3 h was able to prevent a decrease in longevity of the primed seeds (Table 3).

The similarity in these treatments is the exposure of seeds to reduced water content during a period sufficient to allow the necessary processes to proceed. Exceptions to this are the treatments at high temperatures (Fig. 2, Table 2) where a water content equal to the one experienced during priming improved longevity. At the higher moisture contents, close to the priming moisture content, the priming process continues independent of the temperature used, as revealed by the further reduction of t_{50} (Fig. 2). However, the 32°C treatment after priming resulted in an increased longevity, even at 38% moisture content. This suggests that the higher temperature can initiate processes that restore longevity but does not block all germination-related processes. A short heat shock (3 h) might act via the same mechanism, because the heat shock is only effective at the higher moisture contents close to the priming moisture content.

Either a mild water stress or a high temperature stress is effective in enhancing the longevity of primed seeds, and in combination they appeared to have the strongest effect (Table 2, Fig. 2). This is similar to the induction of desiccation tolerance in germinated seeds which could be reinstated by applying water stress to the seeds for several days (Bruggink and van der Toorn, 1995). Here also, high temperatures were effective under certain conditions (unpublished results), although this was never tested in the absence of water stress, because radicle growth would continue in that case.

One of the objectives of priming is the reduction of the time to germination. In general this is correlated with a reduction in longevity. The methods used here to enhance longevity either had little influence on time to germination or even shortened it further, indicating

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that the mechanism is not simply a reversal of the priming effect. Such a reversal was only found at high temperatures in combination with low moisture contents (Fig. 2). Maybe processes leading to germination are negatively affected by such conditions, while processes leading to prolonged longevity still proceed.

It is not clear whether the methods described here are able to improve longevity in primed seeds of every species. Tarquis and Bradford (1992) carried out similar treatments with lettuce seeds where they found no effects of drying conditions after priming on subsequent longevity.

The similarity in induction methods for desiccation tolerance in germinated seeds (Bruggink and van der Toorn, 1995), and for longevity in primed seeds suggests that the same physiological mechanisms are involved. This further supports the idea that desiccation tolerance and longevity are related characteristics.

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