South African Journal of Botany 2003, 69(4): 455–461
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF BOTANY
EISSN 1727–9321

Minireview

Thermoinhibition of seed germination

PN Hills and J van Staden*

Research Centre for Plant Growth and Development, School of Botany and Zoology, University of Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
* *Corresponding author, e-mail: vanstadenj@nu.ac.za*

Received 21 August 2003, accepted in revised form 25 August 2003

Thermoinhibition describes the inability of seeds to germinate at high temperatures, although germination proceeds immediately when the temperature is reduced below a certain threshold level. This phenomenon is distinct from thermodormancy, where some form of dormancy-breaking treatment is required before germination can proceed at the favourable temperature. Like seed dormancy, thermoinhibition is manifested in a number of different ways, ranging from simple high-temperature induced changes to the structures enclosing the embryo which prevent radicle emergence, to the interaction of a number of different factors, and probable expression of certain genes inhibitory to germination which may be temperature regulated. Thermoinhibition occurs in a large number of important crop species, so that an understanding of this phenomenon is both of scientific interest and practical importance.

Introduction

Temperature strongly influences both physiological and biochemical processes, including those occurring in germinating seeds. In the field, temperature acts to regulate germination in three main ways (Bewley and Black 1986). Firstly, it may be involved in the removal of either primary or secondary dormancy. Secondly, temperatures outside of the normal limits for germination may cause the establishment of secondary dormancy in a seed when conditions are not favourable for seedling establishment. Finally, the temperature at which seeds are incubated determines their capacity for germination and the rate at which this occurs.

Each species has a range of temperatures over which germination may proceed and within which seedling establishment is possible (Bradbeer 1988). There are three cardinal temperatures for germination, namely the maximum, minimum and optimum germination temperatures (Bewley and Black 1982). The maximum and minimum temperatures represent the extremes of the range of temperatures over which the seed is able to germinate. The optimum germination temperature may be defined as that temperature at which the highest percentage germination may be obtained in the shortest possible period of time (Mayer and Poljakoff-Mayber 1975). These temperatures vary considerably between species, but may also vary between cultivars of the same species (Bewley and Black 1982) and are thus determined by the source of the seeds, genetic differences within a species and the age of the seed (Mayer and Poljakoff-Mayber 1975). These temperatures may be considerably modified by several other factors, such as exposure to light of different wavelengths or the application of different compounds, including phytohormones such as ethylene, abscisic acid (ABA) and gibberellins.

Within the temperature range of a species, the rate of germination usually increases as the temperature rises, although the final percentage germination may decline (Heydecker 1977). However, as the temperature approaches the maximum for germination, the germination rate begins to slow (Heydecker 1977). Variations in the rates of germination of different seeds ensure that the seeds in a population germinate at different times, thereby leading to the temporal dispersal of germination (Bewley and Black 1986). In wild populations this is a distinct advantage. However, it is disadvantageous in a field crop where uniformity is an important consideration.

In the environment, temperatures greater than the maximum favourable temperature for germination will result in the suspension of germination. At these supra-optimal temperatures, seeds may enter into a state of either thermodormancy or thermoinhibition. It is important that a distinction be made between these two phenomena. In thermodormant seeds, a state of secondary dormancy is induced by exposure to the elevated temperature, which must then be released by some form of dormancy-breaking treatment before the seeds are able to germinate again at their optimal

temperature (Vidaver and Hsiao 1975). Seeds are said to be thermoinhibited where they fail to germinate at a high temperature, but where germination proceeds immediately upon transfer to a temperature suitable for germination of seeds of that species (Horowitz and Taylorson 1983). In both thermodormant and thermoinhibited seeds, if the temperature is too high or is maintained for an extended period, thermal death will result (Horowitz and Taylorson 1983). In certain circumstances, however, this distinction is hard to make and terminology has been a problem in the study of thermoinhibition. Many authors, particularly in earlier papers, refer to thermodormant seeds, when the seeds in fact appear to be thermoinhibited. Vidaver and Hsiao (1975) stated: 'Secondary dormancy is a distinctly different condition from so-called thermodormancy in these seeds. The term thermoinhibition should probably be substituted for thermodormancy since seeds which have not yet become dormant will germinate merely by lowering the temperature.' This is a particular problem in lettuce seed studies. The threshold temperature at which lettuce seeds fail to germinate varies between different cultivars, with some genotypes becoming inhibited at temperatures as low as 25°C (Nascimento *et al*. 2001). When this threshold temperature is exceeded during imbibition, seeds become thermoinhibited. However, if this supra-optimal temperature is maintained for more than approximately 72h, the seeds enter into a state of thermodormancy, such that these seeds will then fail to germinate even when the temperature is reduced below the inhibitory threshold. A similar situation appears to occur in celery seeds, in which seeds that do not germinate at high temperatures will germinate when transferred to a lower temperature. The longer the seeds are incubated at the supra-optimal temperature, the longer they appear to take to germinate when the temperature is reduced (Biddington and Thomas 1978). It is therefore important to examine the actual germination curves of the various seed lots used in the literature to determine whether the seeds used were in fact thermodormant or thermoinhibited.

Observations of temperature effects on the germination of seeds of a number of different species have led to the formulation of a number of hypotheses concerning the basis of thermoinhibition in those species. An analysis of the available literature reveals that there appears to be a variety of possible mechanisms resulting in the arrest of germination. The situation is made more confusing by the fact that there appears to be a complex interplay between several different factors in a number of species. This is particularly evident in lettuce seeds. In this species, not only do different factors appear to be involved in thermoinhibition in different cultivars, but in certain cultivars, a number of factors seem to act synergistically to prevent germination at supra-optimal temperatures. In the cultivar Grand Rapids for instance, thermoinhibition has been proposed to be due to restrictions on radicle expansion by the endosperm or pericarp (Drew and Brocklehurst 1984), by changes in endogenous cytokinin and gibberellin levels (Saini *et al*. 1986), by changes in phytochrome in the seeds (Kristie and Fielding 1994), by abscisic acid (ABA) (Yoshioka *et al*. 1998) and by anaerobic respiration and reduced ATP production (Small *et al*. 1993). Whilst it is important to consider the complex interplay that does exist between these different factors in the various species or genotypes that eventually results in the seed becoming thermoinhibited, for the purposes of this review, each potential factor which has been proposed as playing a role in thermoinhibition will, as far as possible, be dealt with separately.

Involvement of the embryo coverings

In many species, seed dormancy is imposed by the structures surrounding the embryo, including the endosperm, perisperm, pericarp and testa (Bewley and Black 1982). These structures may impose dormancy through a number of mechanisms, including mechanical restraint on embryo extension, the establishment of permeability barriers interfering with water uptake, gaseous exchange or the outward diffusion of endogenous germination inhibitors. Similar mechanisms may also be associated with thermoinhibition in some species.

In the legume *Lathyrus sativus*, supra-optimal temperatures increase hardseededness, preventing seed hydration and germination (Agrawal *et al*. 1980). In spinach (*Spinacia oleracea*) seeds, germination is inhibited at temperatures in excess of 30°C (Leskovar *et al*. 1999). At this temperature, the pericarp appears to act as a physical barrier to germination as well as a source of inhibitory compounds (Leskovar *et al*. 1999). Complete removal of the pericarp was able to overcome thermoinhibition at 30°C, however, placing the pericarp near the embryos had a slight inhibitory effect, which was more pronounced if the embryos were re-inserted into the excised pericarps (Leskovar *et al*. 1999).

The integuments of apple seeds are rich in phenolic compounds that fix oxygen through oxidation, thereby lowering the levels of oxygen available to the embryo (Côme and Tissaoui 1973). When the seeds are imbibed at high temperatures the embryo's requirement for oxygen is increased as a result of an increase in the metabolic rate. The amount of oxygen available to the embryo is, however, decreased as oxidation of phenolics rises and oxygen becomes less soluble in the water in the seed coat and germination is consequently inhibited (Côme and Tissaoui 1973). Hypoxic conditions imposed by the seed coat at supra-optimal temperatures may have a different effect in seeds that require ethylene for germination. Since the ethylene-forming enzyme (ACC oxidase) is oxygen-dependant, the conversion of ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene may be inhibited, preventing germination (Yang 1985).

Embryo coverings also play a role in the thermoinhibition of lettuce seeds at supra-optimal temperatures (Drew and Brocklehurst 1984). The involvement of different tissues surrounding the embryo in thermoinhibition appears to vary between seeds of different cultivars. In cv. Cobham Green lettuce seeds, control over germination appears to be exerted by the pericarp, since relief of thermoinhibition at 35°C resulted when the pericarp was weakened by hypochlorite treatment (Drew and Brocklehurst 1984). Hypochlorite treatment of the pericarp also appeared to overcome thermoinhibition in cultivars Empire and Benita, whilst treatment of seeds of cultivars Sabine and Bellona resulted in faster recovery from thermoinhibition than non-treated seeds

(Drew and Brocklehurst 1987). In seeds of cv. Grand Rapids, however, thermoinhibition was relieved by weakening of the endosperm (Drew and Brocklehurst 1984). Dutta *et al*. (1997) also observed that even minor punctures to the endosperm resulted in complete alleviation of thermoinhibition in cv. Pacific lettuce seeds.

Bradford and Somasco (1994) explained the role of embryo coverings in thermoinhibition in lettuce seeds by analysing water relations in lettuce cv. Empire seeds. This work did not attempt to distinguish between the effect of the endosperm and the pericarp, but rather refers to the effects of the embryo coverings as a whole. The ability of the embryo to absorb water from its surroundings and initiate growth is determined by the water potential (Ψ) of its cells. Thermoinhibition is associated with an increase in the yield threshold that must be exceeded in order for radicle emergence to take place (Karssen *et al*. 1989). This total yield threshold is made up of a component from the radicle cell walls (Y_r) and a component from the tissues surrounding the embryo (Y_e). As the incubation temperature increases, the osmotic potential of the embryo ($\Psi\pi$), the embryo turgor threshold for growth (Y_r) and endosperm resistance (Y_e) all increase relative to seeds incubated at optimal temperatures, causing thermoinhibition (Bradford and Somasco 1994). The removal of the structures enclosing the embryo thus results in the alleviation of thermoinhibition by eliminating the contribution of Y_e to the total yield threshold (Bradford and Somasco 1994). This was also shown in the lettuce cv. Pacific, where slitting the seeds to disrupt the integrity of the endosperm extended the temperature range for germination and maintained lower base water potential in the slit seeds as compared with intact seeds (Dutta and Bradford 1994). The fact that removal or scarification of the endosperm does not affect differences in temperature sensitivity between seeds of different lettuce seed cultivars (Dunlap *et al*. 1990, Prusinski and Khan 1990), however, indicates that the embryo itself also plays a key role in thermoinhibition (Bradford and Somasco 1994).

In lettuce seeds, the protrusion of the radicle through the micropylar endosperm signals the end of germination. Weakening of the endosperm through cell wall autohydrolysis thus may be important for germination in lettuce seeds (Dutta *et al*. 1994). Autolytic activity appeared to be an enzymatic process as it was dependant on temperature and pH (Dutta *et al*. 1994). When isolated cell walls from thermoinhibited or ABA-treated seeds were examined, autohydrolysis was reduced by up to 25% compared with control endosperms isolated from germinating seeds. Treating seeds with kinetin and GA increased the rate of hydrolysis by 20% to 30% compared with thermoinhibited controls (Dutta *et al*. 1994). In a later study (Dutta *et al*. 1997), it was reported that this autohydrolysis was related to a cell-wall bound endo-β-mannanase which was regulated by the same conditions as was germination, such that enzymic activity was almost completely suppressed in thermoinhibited seeds. Endo-β-mannanase activity has since been shown to be higher in thermotolerant than in thermosensitive genotypes, especially at high temperatures (Nascimento *et al.* 2000, 2001). Priming of lettuce seeds increased endo-β-mannanase activity and allowed for 100% germination of the thermosensitive cultivar Dark Green Boston at 35°C, whilst only 4% of non-primed seeds germinated at this temperature (Nascimento *et al.* 2001). These results suggest that enzymatic weakening of the endosperm may play an important role in the germination of certain lettuce seed cultivars.

Gibberellins and cytokinins

From their work on celery (*Apium graveolens*) cv. Lathom Blanching, Biddington and Thomas (1978) reported that temperature may control germination in these seeds by affecting endogenous levels of cytokinins and gibberellins. Exogenous application of these hormones increased the germination of celery seeds subjected to supra-optimal temperatures in both the light and the dark. These two hormones also appeared to act in a synergistic manner in the dark (Biddington and Thomas 1978). A similar result has also been shown for the lettuce cv. Grand Rapids (Saini *et al*. 1986). Biddington and Thomas (1978) proposed that temperature may control germination in celery seeds by affecting the levels of endogenous gibberellins or a gibberellic acid/protein complex. They suggested that germination is primarily induced by gibberellins, endogenous levels of which decline as temperatures increase and rise again when the temperature is reduced.

Relief from thermoinhibition was enhanced by white or red light (Biddington and Thomas 1978). Quantitative and qualitative changes in the cytokinin contents of seeds have been reported in response to dormancy-breaking red light treatment of celery, lettuce (Van Staden 1973) and *Rumex obtusifolius* (Van Staden and Wareing 1972). These results suggest that phytochrome-mediated control over the germination process is expedited via endogenous cytokinins. Biddington and Thomas (1978) therefore suggested that red-light may enhance the effect of gibberellins on the relief of thermoinhibition through increased cytokinin levels.

Cytokinins may play a subsidiary role in alleviating thermoinhibition in lettuce seeds by increasing ethylene biosynthesis or the sensitivity of the seeds to ethylene. Exogenously applied kinetin and ACC act synergistically to increase pre-germination levels of ethylene and enhance germination at temperatures of 32°C and 35°C (Khan and Prusinski 1989). Removing the restriction imposed by the tissues enclosing the embryo by cutting the testa allowed ACC to be readily taken up and converted to ethylene, removing the synergistic effect of the added kinetin. This result suggests that cytokinins have an important regulatory role for ethylene biosynthesis and hence for germination in intact lettuce seeds at high temperatures, and that the seed coat may be an important factor in this regulation (Khan and Prusinski 1989). This may involve enhanced utilisation of ACC as a result of activation of the ethylene-forming enzyme (EFE) or ACC oxidase (Huang and Khan 1992) and an interaction of the ACC-derived ethylene with the cytokinin (Khan and Prusinski 1989). In lettuce cv. Grand Rapids, treatment with kinetin and oxygen also stimulated germination at thermoinhibitory temperatures, possibly either by causing seeds to bypass their ethylene requirement or increasing their sensitivity to ethylene (Small *et al*. 1993).

Ethylene

In a number of species, seed germination is strongly related to the biosynthesis and action of ethylene (Gallardo *et al*. 1996). Huang and Khan (1992) found that pre-conditioning seeds of the lettuce cv. Mesa 659 with the moist solid carrier Micro-Cel E conferred greater thermotolerance on these seeds, alleviating thermoinhibition at 35°C. The ability of the pre-conditioned seeds to rapidly synthesise ACC and to respond to ACC and ethylene on exposure to temperatures which were normally thermoinhibitory appeared to be the critical factor for the alleviation of thermoinhibition. Exposing seeds to aminoethoxyvinylglycine (AVG), an inhibitor of ACC synthesis, prevented pre-conditioned seeds from germinating at 35°C. This inhibitory effect of AVG was reversed by supplying the seeds with exogenous ACC, ethephon or ethylene (Huang and Khan 1992). The use of ACC is enhanced by cytokinins under conditions of temperature stress (Khan and Huang 1988), and the production of cytokinin or cytokinin-like factors may also play a regulatory role in ethylene biosynthesis during thermoinhibition (see above) (Huang and Khan 1992).

Alleviation of thermoinhibition in chickpea (*Cicer arietinum*) may also be induced by the addition of exogenous ethylene or by the stimulation of ethylene production within the seeds (Gallardo *et al*. 1996). Thus the addition of ethrel (chloroethylphosphonic acid), ACC or spermine to the germination medium or the inhibition of polyamine biosynthesis, which competes with the ethylene biosynthetic pathway for the same precursors, by CHA (cyclohexamine) or MGBG (methyl-glyoxal-*bis*-guanylhydrazone), could induce germination of chickpea seeds at thermoinhibitory temperatures (Muñoz De Rueda *et al*. 1994).

Unlike lettuce seeds, where high temperatures inhibit ACC production, supra-optimal temperatures appear to stimulate ACC synthase activity in chickpea seeds. Despite this stimulation, ACC levels decrease in thermoinhibited seeds possibly due to an increase in ACC conjugation (Gallardo *et al*. 1991). With an increase in temperature, a decrease in the levels of ACC in the embryonic axis of chickpea seeds and a concomitant increase in the levels of conjugated ACC in the embryonic tissues was observed. Furthermore, malonyl ACC-transferase, the enzyme responsible for the conjugation of ACC, has a very low K_m value. It therefore appears that, in chickpea seeds, ACC malonisation and the consequent inhibition of ethylene synthesis is one of the principle causes of thermoinhibition (Gallardo *et al*. 1996). The conversion of ACC to ethylene may also be affected at high temperatures, possibly through some temperature effect on ACC oxidase (Gallardo *et al*. 1996). In sunflower (*Helianthus annuus*) too, thermoinhibition is associated with the loss of the seed's ability to synthesise ethylene (Corbineau *et al*. 1989).

Besides changes in ethylene biosynthesis, ethylene and ethylene action have also been implicated in the alleviation of stress on lettuce seed germination (Prusinski and Khan 1990). Saini *et al*. (1989) have reported that in lettuce seeds, thermoinhibition does not appear to be related only to a reduction in the ability of the seeds to synthesise ethylene, but may rather be due to high temperatures causing an increase in the threshold concentrations of this hormone required to elicit germination. Prusinski and Khan (1990) have also reported that seeds and seedlings have a greater sensitivity to ethylene when stressed than under non-stressful conditions, which may suggest a greater need for this phytohormone for growth processes under stress conditions. These authors found a positive correlation between the ethylene-producing capacity of seeds of a number of lettuce cultivars and the ability of the seeds to germinate under conditions of salinity, osmotic and temperature stress. Nascimento *et al*. (2000) also observed a correlation between thermosensitivity and ethylene production in lettuce at high temperatures, with thermotolerant cultivars such as Everglades and PI251245 producing more ethylene than thermosensitive cultivars such as Dark Green Boston and Valmaine at 35°C.

The effect of ethylene in overcoming thermoinhibition appears to be linked fairly closely with other metabolic changes within lettuce seeds at supra-optimal temperatures, including changes in phytochrome (Saini *et al*. 1989). Ethylene, GA_3, kinetin, carbon dioxide (CO_2) and light were all shown to have no effect on overcoming thermoinhibition at 32°C, but that the action of any one of the hormones required the presence of at least one other growth regulator, light or CO_2 or a combination of these. To achieve 100% germination, at least three stimuli were required (Saini *et al*. 1986). Nascimento *et al*. (2000) also observed an interaction between ethylene and light. This suggests that thermoinhibition in relation to ethylene may be regulated at a number of points.

The mechanism whereby ethylene is involved in the regulation of germination appears therefore to differ in different species of plants. It should, however, be noted that the alleviation of thermoinhibition by ethylene is limited to species in which seed germination is dependent on that phytohormone.

Abscisic acid

Fluoridone (1-methyl-3-phenyl-5-[3-trifluorylmethyl-(phenyl)]-4-(1*H*)-pyridinone) is an inhibitor of the carotenoid biosynthetic pathway enzyme phytoene desaturase. Since carotenoids are the main precursors of abscisic acid (ABA) in plants, inhibition of carotenogenesis also inhibits ABA biosynthesis (Yoshioka *et al*. 1998). Application of this compound to lettuce cv. Grand Rapids was able to overcome thermoinhibition up to 33°C, although at this temperature germination was markedly delayed as compared to 23°C (Yoshioka *et al*. 1998). Fluoridone application also overcame inhibition of germination at supra-optimal temperatures in seeds of *Chrysanthemum parthenim*, *Freesia hybrida*, *Cerastium glomeratum*, *Stellaria neglecta*, *Agrostis alba*, *Conyza canadensis*, *Cryptotaenia japonica*, *Dactylis glomerata*, *Festuca rubra*, *Medicago sativa*, *Plantago lanceolata*, *Trifolium repens* and *Vicia angustifolia*, although the range of temperatures at which fluoridone promoted germination differed between species (Yoshioka *et al*. 1998). Fluoridone had no effect on two gramineous weeds, *Bromus catharticus* and *Lolium perenne*, which were primarily dormant as opposed to thermoinhibited. These authors therefore sug-

gested that ABA plays a decisive role in the regulation of seed germination at supra-optimal temperatures.

Repression of metabolic reactivation

In *Phacelia tanacetifolia* seeds, germination is inhibited at temperatures in excess of 26°C (Pirovano *et al*. 1997). This inhibition is correlated with the lack of activation of metabolic processes within the seed. In the first 24h of imbibition, an increase in the levels of glucose-6-phosphate (Glc-6-P) was inhibited first, followed by inhibition of increases in enzymic activity and then by inhibition of increases in levels of ATP, reducing sugars, RNA and DNA. The inhibition of radicle emergence correlated most strongly with the levels of Glc-6-P. After 24h, when germination was inhibited by 60%, only the level of Glc-6-P was still inhibited to the same extent (Pirovano *et al*. 1997). Appreciable qualitative differences in transcriptional and translational activities were detected at 16°C and 30°C. Both activities were higher in seeds incubated at 30°C than in seeds incubated at the lower temperature after 9h, but with time the translational activities became much lower at 30°C than at 16°C (Pirovano *et al*. 1997). Examination of protein patterns also revealed that a number of polypeptides which disappeared during imbibition at 16°C did not disappear in temperature-inhibited seeds (Pirovano *et al*. 1997).

High temperatures may inhibit germination at two levels (Pirovano *et al*. 1997). Firstly, the high temperature may have a general effect on the rate of the reactivation processes occurring within the imbibing seeds. This may be linked to the fact that cells of seeds incubated at 30°C also had lowered levels of mobile potassium (K^+) ions and the plasma membranes showed greater permeability to K^+, compared with cells of seeds incubated at 16°C. The control of K^+ concentrations in cells is crucial for the regulation of metabolic activity and these authors suggested that reduced cellular concentrations of K^+ may inhibit the activation of K^+-dependant mechanisms and hence germination in seeds at 30°C. Secondly, elevated temperatures may also work directly on single reactions involved in the inhibitory mechanism of germination in *P. tanacetifolia* seeds, either inducing a lack of function through denaturation of key components or by increasing processes related to the synthesis of inhibitory substances.

In lettuce cv. Grand Rapids, thermoinhibition at 38°C markedly reduced ATP and total adenylate content of the seeds, as well as adenylate energy charge (Small *et al*. 1993). Seeds had a high ethanol content at this temperature and appeared to undergo ethanolic fermentation, probably as a result of a reduction in oxygen solubility at this temperature. Thermoinhibition was alleviated and seeds contained normal levels of ATP when treated with 100% oxygen and kinetin at 38°C (Small *et al*. 1993). A reduction in aerobic respiration and consequently in ATP production may therefore be a contributing factor in thermoinhibition (Small *et al*. 1993).

The involvement of a thermo-labile factor

Before Pirovano *et al*. (1997) proposed that denaturation may be a factor in thermoinhibition, Takeba and Matsubara (1976) reported that a thermo-labile process is involved in the germination of lettuce cv. New York seeds. Germination was completely inhibited at a temperature of 30°C in the dark, but was re-activated after incubation at 20°C. Although Takeba and Matsubara did not establish the nature of the thermo-labile factor, they proposed that it was probably protein-based. The longer the period that the seeds were pre-incubated at 30°C, the longer they required at the lower temperature before germination occurred. A similar trend has been observed in *Tagetes minuta*, where seeds incubated for extended periods of more than 20 days at 35°C begin to take longer to achieve germination (Forsyth and Van Staden 1983). Takeba and Matsubara suggested that this thermo-labile factor was thermoreversible and that the degree of its re-activation upon return to favourable temperatures is dependant on the extent of its inactivation. Three models were proposed to attempt to interpret these results in terms of the responses of the lettuce seeds to light and temperature (Takeba and Matsubara 1976):

1 Active phytochrome may promote germination by increasing the amount of the precursor of the reaction mediated by the thermo-labile factor,
2 The product(s) of the phytochrome system is(are) changed to the essential metabolite for germination by combining with the product(s) of the reaction mediated by the thermo-labile factor, and
3 The phytochrome system increases the stability of the thermo-labile factor by unknown mechanisms.

Phytochrome

In lettuce, the promotion of seed germination through the red/far-red reversible or Low Fluence Response (LFR) is closely linked to temperature (Kristie and Fielding 1994). For the majority of lettuce cultivars, a pulse of red light is able to promote germination within a certain range of temperatures. When this range is exceeded, the response to the red light pulse is diminished and seeds become thermoinhibited (Vidaver and Hsiao 1975). In lettuce cv. Grand Rapids, dark germination at 20°C results in almost 100% germination, but thermoinhibition prevents germination at 27°C and above (Saini *et al*. 1989). This has been attributed to the reversion of Pfr to Pr before it has time to produce an effect, a hypothesis which is supported by the finding that repeated red light pulses raise the upper threshold temperature for germination by several degrees as compared to a single pulse of red light (Saini *et al*. 1989). Kristie and Fielding (1994) suggested that dark germination was governed by levels of Pfr and that the mean level of Pfr required to induce dark germination was increased as temperatures rose. It was proposed that this may be attributable to an increase in dark-reversion of Pfr to Pr at high temperatures (Kristie and Fielding 1994). Preliminary Pfr requirement curves generated from a number of different lettuce cultivars suggest that the shape of the Pfr requirement curve may partially account for differences in the temperature response curves for dark germination that occur in the different cultivars (Kristie and Fielding 1994).

The alleviatory effect of the phytochrome system at high

temperatures may, as previously mentioned, be mediated through an effect on the levels of various phytohormones required for germination, including ethylene (Saini *et al*. 1986, 1989, Nascimento *et al*. 2000) and cytokinins (Biddington and Thomas 1978). Nascimento *et al*. (2000) also observed that endo-β-mannanase activity was eliminated when lettuce seeds were imbibed in the dark at 35°C, thus the activity of this enzyme may also in some manner be mediated through the phytochrome system.

Gene expression

From the above, it would seem that in all cases described thus far in the literature, thermoinhibition is a passive state, imposed on seeds held under conditions of temperature stress through heat-induced changes in their normal physiological functioning. Whilst most studies have focussed on specific aspects of seed physiology, such as plant growth regulators, enzymic reactions or phytochrome, it would appear that control of germination is levied at multiple sites and that thermoinhibition is actually the result of a complex interaction of a number of factors in these species.

In *Tagetes minuta* achenes, which have an optimum germination temperature of 25°C, temperatures in excess of 35°C result in the achenes becoming thermoinhibited. If the temperature is reduced even slightly below 35°C, germination proceeds rapidly (Drewes 1989). None of the factors described in the literature appear to be involved in thermoinhibition in this species. The achenes easily take up water when imbibed at 35°C. Whilst the seeds have a light requirement for germination, seeds incubated at 35°C in white light become thermoinhibited. Ethylene does not appear to be involved in thermoinhibition in *T. minuta* achenes. The application of ethrel to achenes imbibed at 35°C, either as short pulses or through continuous exposure, had no statistically significant alleviatory effect on thermoinhibition of achenes of this species (Drewes 1989). When ethrel was applied at 25°C, the germination rate was not affected, indicating that *T. minuta* achenes are not dependant on ethylene for their germination (Drewes 1989). Indeed, ethrel treatment of the achenes resulted in abnormal post-germinative development and both hypocotyl and radicle extension were severely retarded. The cytokinins kinetin and benzyladenine (BA) also had no effect on thermoinhibited achenes (Drewes 1989). No alleviatory effect was observed when achenes were treated with a commercially available mixture of gibberellin GA_{4+7} and BA (Promalin), indicating that even a combination of light and multiple growth regulators was unable to prevent thermoinhibition in this species. The application of gibberellins alone, however, did partially alleviate thermoinhibition in this species, with GA_{4+7} being more effective than GA_3 (Drewes 1989). Yet even GA application was unable to induce more than approximately 75% germination at 35°C and no major changes in endogenous gibberellin-like substances were detected in thermoinhibited achenes (Drewes 1989).

When protein expression in germinating and thermoinhibited achenes was compared using two-dimensional polyacrylamide gel electrophoresis, ten polypeptides were identified from the thermoinhibited achenes which were not expressed at any stage during germination of the achenes at 25°C (Hills *et al*. 2001). These polypeptides all disappeared rapidly when the temperature was reduced and the achenes allowed to germinate, in a manner which correlated tightly with an increase in the germinability of the achenes. Concomitant with the decline of these thermoinhibition-associated polypeptides, a number of other proteins which were not synthesised during thermoinhibition and which are presumably involved in germination and in post-germinative events were produced (Hills *et al*. 2001). This suggests that thermoinhibition in this species may be imposed in a manner not previously described, possibly by an actively-imposed block on germination through gene expression. Thus, these ten thermoinhibition-associated polypeptides may be involved in actively repressing germination at temperatures which are not conducive to seedling establishment, in a manner similar to the inhibition of precocious germination or to genetically-imposed seed dormancy.

Conclusions

Whilst this paper has dealt with each of the many factors which appear to cause thermoinhibition in different species separately, it is important to remember that in many species several different factors may be involved in causing the suspension of germination and that these factors may interact in exceedingly complex ways. To attempt to explore all these interactions is beyond the scope of this review.

Thermoinhibition is a particularly interesting and important part of seed biology. It differs from thermodormancy in that no dormancy breaking treatment is required before germination is able to continue. This occurs as soon as the temperature at which the seeds are incubated has fallen to levels which favour germination for that particular species. Since many of the factors which have been proposed to cause thermoinhibition are the same or are similar to those which are implicated in seed dormancy, thermoinhibition, like dormancy provides an opportunity to examine processes in germinating seeds which are able to interrupt the germination process and thus to gain greater insight into germination itself. Unlike dormancy, however, thermoinhibition allows the unique possibility of then allowing the seeds to continue germinating without any need for treatments which may in some way interfere with the experimental situation.

Acknowledgements — The authors wish to acknowledge the financial support of the National Research Foundation of South Africa and the University of Natal Pietermaritzburg.

References

Agrawal PK, Sethi KL, Mehra RB (1980) Effect of temperature on germination of *Lathyrus sativus* seeds: An analysis. *Seed Research* **8**: 25–32

Bewley JD, Black M (1982) Physiology and Biochemistry of Seeds in Relation to Germination, Vol 2. Springer-Verlag, Berlin, pp 297, 304

Bewley JD, Black M (1986) Seeds: Physiology of Development and Germination, Vol 2. Springer-Verlag, Berlin, pp 297, 304

Biddington NL, Thomas TH (1978) Thermodormancy in celery seeds and its removal by cytokinins and gibberellins. *Physiologia Plantarum* **42**: 401–405

Bradbeer JW (1988) Seed Dormancy and Germination. Blackie, London, pp 1–3, 6

Bradford KJ, Somasco OA (1994) Water relations of lettuce seed thermoinhibition. I. Priming and endosperm effects on base water potential. *Seed Science Research* **4**: 1–10

Côme D, Tissaoui T (1973) Interrelated effects of imbibition, temperature and oxygen on seed germination. In: Heydecker W (ed) Seed Ecology. Butterworths, London, pp 157–168

Corbineau F, Rudnicki RM, Côme D (1989) ACC conversion to ethylene by sunflower seeds in relation to maturation, germination and thermodormancy. *Plant Growth Regulation* **8**: 105–115

Drew RLK, Brocklehurst PA (1984) Investigations on the control of lettuce seed germination at high temperatures. *Journal of Experimental Botany* **35**: 986–993

Drew RLK, Brocklehurst PA (1987) The effect of sodium hypochlorite on thermo-inhibition of lettuce seed. *Acta Horticulturae* **215**: 179–184

Drewes FE (1989) An Examination of the Thermoinhibition of *Tagetes minuta* L. Achenes. MSc Thesis, University of Natal Pietermaritzburg, Pietermaritzburg, South Africa

Dunlap JR, Scully BT, Reyes DM (1990) Seed coat-mediation of lettuce germination responses to heat and sodium chloride. *Journal of the Rio Grande Valley Horticultural Society* **43**: 55–61

Dutta S, Bradford KJ (1994) Water relations of lettuce seed thermoinhibition. II. Ethylene and endosperm effects on base water potential. *Seed Science Research* **4**: 11–18

Dutta S, Bradford KJ, Nevins DJ (1994) Cell-wall autohydrolysis in isolated endosperms of lettuce (*Lactuca sativa* L.). *Plant Physiology* **104**: 623–628

Dutta S, Bradford KJ, Nevins DJ (1997) Endo-β-mannanase activity present in cell wall extracts of lettuce endosperm prior to radicle emergence. *Plant Physiology* **113**: 155–161

Forsyth C, Van Staden J (1983) Germination of *Tagetes minuta* L. I. Temperature effects. Annals of Botany **52**: 659–666

Gallardo M, Del Mar Delgardo M, Sanchez-Calle I, Matilla AJ (1991) Ethylene production and 1-aminocyclopropane-1-carboxylic acid conjugation in thermoinhibited *Cicer arietinum* L. seeds. *Plant Physiology* **97**: 122–127

Gallardo M, Sanchez-Calle I, Muñoz De Rueda P, Matilla AJ (1996) Alleviation of thermoinhibition in chickpea seeds by putrescine involves the ethylene pathway. Australian *Journal of Plant Physiology* **23**: 479–487

Heydecker W (1977) Stress and seed germination: An agronomic view. In: Khan AA (ed) The Physiology and Biochemistry of Seed Dormancy and Germination. North Holland, Amsterdam, pp 237–282

Hills PN, Viljoen CD, Van Staden J (2001) Differences in polypeptide expression in thermoinhibited and germinating achenes of *Tagetes minuta* L. Plant Growth Regulation **34**: 187–194

Horowitz M, Taylorson RB (1983) Effect of high temperatures on imbibition, germination, and thermal death of velvetleaf (*Abutilon theophrasti*) seeds. *Canadian Journal of Botany* **61**: 2269–2276

Huang XL, Khan AA (1992) Alleviation of thermoinhibition in preconditioned lettuce seeds involves ethylene, not polyamine biosynthesis. *Journal of the American Society of Horticultural Science* **117**: 841–845

Karssen CM, Haigh A, Van der Toorn P, Weges R (1989) Physiological mechanisms involved in seed priming. In: Taylorson RB (ed) Recent Advances in the Development and Germination of Seeds. Plenum Press, New York, pp 269–280

Khan AA, Huang XL (1988) Synergistic enhancement of ethylene production and germination with kinetin and 1-aminocyclopropane-1-carboxylic acid in lettuce seeds exposed to salinity stress. *Plant Physiology* **87**: 847–852

Khan AA, Prusinski J (1989) Kinetin enhanced 1-aminocyclopropane-1 carboxylic acid utilization during alleviation of high temperature stress in lettuce seeds. *Plant Physiology* **91**: 733–737

Kristie DN, Fielding A (1994) Influence of temperature on the Pfr level required for germination in lettuce cv. Grand Rapids. *Seed Science Research* **4**: 19–25

Leskovar DI, Esensee V, Belenfant-Miller H (1999) Pericarp, leachate, and carbohydrate involvement in thermoinhibition of germinating spinach seeds. *Journal of the American Society for Horticultural Science* **124**: 301–306

Mayer AM, Poljakoff-Mayber A (1975) The Germination of Seeds, Vol 2. Pergammon, Oxford, pp 32–35

Muñoz De Rueda PM, Gallardo I, Sanchez-Calle P, Matilla AJ (1994) Germination of chickpea seeds in relation to manipulation of the ethylene pathway and polyamine biosynthesis by inhibitors. *Plant Science* **97**: 31–37

Nascimento WM, Cantliffe DJ, Huber DJ (2000) Thermotolerance in lettuce seeds: Association with ethylene and endo-β-mannanase. *Journal of the American Society for Horticultural Science* **125**: 518–524

Nascimento WM, Cantliffe DJ, Huber DJ (2001) Endo-β-mannanase activity and seed germination of thermosensitive and thermotolerant lettuce genotypes in response to seed priming. *Seed Science Research* **11**: 255–264

Pirovano L, Morgutti S, Espen L, Cocucci SM (1997) Differences in the reactivation process in thermosensitive seeds of *Phacelia tanacetifolia* with germination inhibited by high temperature (30°C). *Physiologia Plantarum* **99**: 211–220

Prusinski J, Khan AA (1990) Relationship of ethylene production to stress alleviation in seeds of lettuce cultivars. *Journal of the American Society of Horticultural Science* **115**: 294–298

Saini HS, Bassi PK, Consolacion ED, Spencer MS (1986) Interactions among plant hormones, carbon dioxide, and light in the relief of thermoinhibition of lettuce seed germination: Studies in a flow-through gaseous system. *Canadian Journal of Botany* **64**: 2322–2326

Saini HS, Consolacion ED, Bassi PK, Spencer MS (1989) Control processes in the induction and relief of thermoinhibition in lettuce seed germination. *Plant Physiology* **90**: 311–315

Small JGC, Schultz C, Cronje E (1993) Relief of thermoinhibition in Grand Rapids lettuce seeds by oxygen plus kinetin and their effects on respiration, content of ethanol and ATP and synthesis of ethylene. *Seed Science Research* **3**: 129–135

Takeba G, Matsubara S (1976) Analysis of temperature effects on the germination of New York lettuce seeds. *Plant and Cell Physiology* **17**: 91–101

Van Staden J (1973) Changes in endogenous cytokinins of lettuce seed during germination. *Physiologia Plantarum* **28**: 222–227

Van Staden J, Wareing PF (1972) The effect of light on endogenous cytokinin levels in seeds of *Rumex obtisifolius*. *Planta* **104**: 126–133

Vidaver W, Hsiao AI (1975) Secondary dormancy in light-sensitive lettuce seeds incubated anaerobically or at elevated temperature. *Canadian Journal of Botany* **53**: 2557–2560

Yang SF (1985) Biosynthesis and action of ethylene. *HortScience* **20**: 41–45

Yoshioka T, Endo T, Satoh S (1998) Restoration of seed germination at supraoptimal temperatures by fluoridone, an inhibitor of abscisic acid biosynthesis. *Plant Cell Physiology* **39**: 307–312

Edited by TH Thomas