Seed Science Research (2003) **13**, 17–34 DOI: 10.1079/SSR2002121

INVITED REVIEW

Functions and regulation of β-1,3-glucanases during seed germination, dormancy release and after-ripening

Gerhard Leubner-Metzger*

Institut für Biologie II, Albert-Ludwigs-Universität, Schänzlestr. 1, D-79104 Freiburg i. Br., Germany

Abstract

β-1,3-Glucanase (βGlu) expression in seeds plays important roles in the regulation of seed germination, dormancy and in the defence against seed pathogens. A thick β-1,3-glucan layer is typical for the seed envelope of cucurbitaceous species, confers seed semipermeability and is degraded during germination. In many species with coat-imposed dormancy, the seed envelope confers a physical constraint to radicle emergence. In the solanaceous species, the micropylar endosperm and testa have this function, and endosperm weakening appears to be a prerequisite for germination. Class I βGlu is transcriptionally induced in the micropylar endosperm of tobacco, tomato and other solanaceous seeds just prior to radicle emergence. βGlu induction and germination are tightly linked in response to plant hormones and environmental factors, e.g. they are both promoted by gibberellins and inhibited by abscisic acid (ABA). Sense and antisense transformation of tobacco reveals two sites of βGlu action: after-ripening-mediated release of testa-imposed dormancy and endosperm rupture during germination. The use of an ABA-inducible chimeric sense-transgene resulted in overexpression of class I βGlu in seeds and provided direct evidence that βGlu contributes to endosperm rupture. A model integrating βGlu, seed dormancy, after-ripening and germination is presented, and possible mechanisms for βGlu action are discussed. It is proposed that βGlu not only helps defend seeds against pathogens, but is also a key factor in regulating coat-imposed dormancy and germination of seeds in response to environmental and hormonal cues.

*Correspondence
Fax: +49–761–2032612
Email: leubner@uni-freiburg.de
Web site: 'The Seed Biology Place' http://www.leubner.ch

Abbreviations: ABA = abscisic acid; DAP = days after pollination; ERE = ethylene-responsive element; GA = gibberellin; βGlu = β-1,3-glucanase; βGlu I = class I β-1,3-glucanase; Gus = β-glucuronidase; PR = pathogenesis-related; TMV = tobacco mosaic virus.

Keywords: abscisic acid, after-ripening, gibberellin, β-1,3-glucanase, *Nicotiana* seeds, seed dormancy, seed germination

Introduction

One of the most intriguing innovations during the evolution of vascular plants has been the ability to form seeds as propagation and dispersal units. The genetic, physiological and biochemical properties of seeds are of utmost importance for the survival of a wild plant species in an ecosystem, and are also critical for seed quality and agricultural yield of crop plants. Relatively little is known about the interconnected molecular key processes regulating seed germination and dormancy in response to plant hormones and environmental cues. The process of germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expansion growth, and usually culminates in rupture of the covering layers and emergence of the radicle, generally considered as the completion of germination (reviews: Hilhorst, 1995; Bewley, 1997b; Li and Foley, 1997; Koornneef *et al.*, 2002). Seed dormancy can be 'coat-imposed' and/or determined by the embryo itself, and is a temporary failure or block of a viable seed to complete germination under physical conditions that normally favour the process. Radicle emergence during seed germination depends on embryo expansion, which is a growth process driven by water uptake. DNA synthesis and cell division are not required and regulation of embryo growth potential appears to be mainly by changes in cell-wall extensibility.

In many plant species with coat-imposed dormancy, the seed envelope also imposes a physical constraint to radicle protrusion, which has to be overcome by the growth potential of the embryo. These covering layers may include diploid, entirely maternal tissues, e.g. testa (seed coat) and

perisperm; and the endosperm, which is triploid in angiosperms, with two-thirds of its genome originating from the mother plant. Endosperm rupture is the main germination-limiting process in members of the *Asteraceae* (e.g. lettuce) and *Solanaceae* (e.g. tomato, tobacco, pepper and *Datura* spp.) and endosperm weakening, a decline in the mechanical resistance of the micropylar endosperm, seems to be necessary for germination to be completed. Ikuma and Thimann (1963) proposed for lettuce that 'the final step in the germination control process is the production of an enzyme whose action enables the tip of the radicle to penetrate through the coat'. Experiments to identify this enzyme(s) have been conducted in a variety of species, and included the analyses of numerous cell-wall modifying proteins, e.g. endo-β-mannanase, β-mannosidase, α-galactosidase, cellulase, pectin methylesterase, polygalacturonase, xyloglucan endo-transglycosylase, β-1,3-glucanase, chitinase, peroxidase and expansin (e.g. Bewley, 1997a; Welbaum *et al.*, 1998; Amaya *et al.*, 1999; Chen and Bradford, 2000; Leubner-Metzger and Meins, 2000; Ren and Kermode, 2000; Mo and Bewley, 2002). Several of these studies provided evidence for the possible contribution of a specific cell-wall hydrolase in a certain species, and unravelled some of the complexity of the hormonal regulation of seed germination and dormancy. However, conclusive evidence for a single 'germination enzyme' has not yet been found. The work on endo-β-mannanase has been summarized in an excellent review by Bewley (1997a), and there is strong evidence that this enzyme is involved in endosperm weakening. While endo-β-mannanase appears to be necessary for tomato endosperm weakening, it is not sufficient for the completion of germination (e.g. Toorop *et al.*, 1996; Still and Bradford, 1997; Wu *et al.*, 2000). Abscisic acid (ABA) clearly controls the final step of radicle protrusion, but it does not inhibit tomato endosperm weakening caused by endo-β-mannanase (e.g. Nonogaki *et al.*, 2000; Toorop *et al.*, 2000; Wu *et al.*, 2000). In the case of tobacco, we proposed that class I β-1,3-glucanase, which is induced in the micropylar endosperm just prior to its rupture and is tightly linked with altered endosperm rupture in response to light, gibberellins (GA), ABA and ethylene, is involved in the regulation of germination (Leubner-Metzger and Meins, 1999). Taken together, these findings support the view that germination control by the seed-covering layers is achieved by the collaborative or successive action of several cell-wall-modifying proteins. Considerable evidence suggests that β-1,3-glucanase substantially contributes to the regulation of germination, dormancy release and after-ripening of dicot seeds, which is the focus of this review.

Structure, regulation and functions of plant β-1,3-glucanases

The β-1,3-glucanases (βGlu; EC 3.2.1.39) are abundant, highly regulated enzymes, widely distributed in seed-plant species (reviews: Meins *et al.*, 1992; Simmons, 1994; Høj and Fincher, 1995; Leubner-Metzger and Meins, 1999; Hrmova and Fincher, 2001; http://www.leubner.ch/). They are able to catalyse hydrolytic cleavage of the 1,3-β-D-glucosidic linkages in β-1,3-glucans, and most of the known plant βGlu are endo-type enzymes. It is suggested that their original role in evolution probably was to promote cell growth and division of unicellular organisms by turning over cell-wall β-1,3-glucans. Overexpression of a plant βGlu in recombinant yeast causes growth inhibition and defects in cell-wall formation (Demolder *et al.*, 1993). βGlu are induced in response to the infection of plants with pathogens and are, therefore, grouped among the pathogenesis-related (PR) proteins as the PR-2 family. Although the major interest in βGlu stems from their role in plant defence against pathogens, there is strong evidence that these enzymes are also implicated in diverse physiological and developmental processes in the uninfected plant, including cell division (Scherp *et al.*, 2001), microsporogenesis (Worrall *et al.*, 1992; Bucciaglia and Smith, 1994), pollen germination and tube growth (Meikle *et al.*, 1991; Doblin *et al.*, 2001), fertilization (Ori *et al.*, 1990), embryogenesis (Helleboid *et al.*, 2000), seed development (Buchner *et al.*, 2002), seed germination (this review), mobilization of storage reserves in the endosperm of cereal grains (Høj and Fincher, 1995; Hrmova and Fincher, 2001), bud dormancy (Krabel *et al.*, 1993; Rinne *et al.*, 2001), and responses to wounding, cold, ozone and ultraviolet light (UVB) (Leubner-Metzger and Meins, 1999). The βGlu exist as a family of multiple isoforms that differ in size, isoelectric point, primary structure, cellular localization and pattern of regulation. The relationships between structure and enzymatic mechanisms of βGlu have been studied in great detail with the cereal isoforms, and are covered by excellent reviews (e.g. Simmons, 1994; Høj and Fincher, 1995; Hrmova and Fincher, 2001).

The major focus of the present review is on βGlu in dicot seeds. The most detailed sequence information for the dicot βGlu is available from cDNA and genomic clones of solanaceous species (for references, see Simmons, 1994; Leubner-Metzger and Meins, 1999). The various βGlu of the genus *Nicotiana* have been classified into four structural classes that differ by a minimum of 40–50% in amino-acid sequence identity of the mature proteins. Similar isoforms have been reported for tomato, pepper, potato and other plant species. The endo-type class I

enzymes (βGlu I) constitute the PR-2e subgroup of the PR-2 family and include the highly homologous 33 kDa basic tobacco isoforms Gla and Glb and the 35 kDa tomato isoform GluB, which shares approximately 90% amino-acid identity with tobacco Gla and Glb. The tobacco and tomato βGlu I proteins are basic intracellular isoforms that contain a characteristic carboxy-terminal extension known to mediate targeting to the vacuole. There is considerable evidence that vacuolar proteins, including class I βGlu and chitinases, can also be secreted (e.g. Kunze *et al.*, 1998; Rinne *et al.*, 2001). In contrast to βGlu I, the class II and III members of the PR-2 family do not contain the carboxy-terminal extension and are secreted into the apoplast. The known tobacco class II and III βGlu are acidic proteins, ranging in apparent size from *c*. 34 to 36 kDa in denaturing gels. Class II also includes the two 41 kDa stylar βGlu isoforms, Sp41a and Sp41b, which are expressed exclusively in the style of tobacco flowers. Sp41a and Sp41b do not appear to be induced by pathogen infection and, hence, are referred to as 'PR-like proteins'. One apoplastic class II isoform (GluA) and two closely related class III isoforms are known from tomato. The class IV βGlu Tag1 is a 'PR-like' protein that is expressed specifically in tobacco anthers and seems to be secreted to degrade the callose wall of the tetrads during pollen grain development.

The βGlu of the PR-2 family are highly regulated in response to developmental, hormonal, environmental and pathogenesis-related factors (for references, see Simmons, 1994; Leubner-Metzger and Meins, 1999). βGlu I accumulate at high concentrations in the roots and in lower leaves of mature, healthy tobacco plants. βGlu I gene expression is transcriptionally induced by ethylene and down-regulated by ABA. In general, the tobacco class II βGlu do not appear to accumulate in vegetative tissues of mature, healthy tobacco plants. Ethylene does not appreciably affect the expression of class II and class III βGlu in leaves of tobacco and tomato. In general, βGlu and chitinases are co-induced in plants infected with viral, bacterial, and fungal pathogens and in response to elicitors, including fungal glucans, linear β-1,3-glucan, chitosan, *N*-acetylchitooligosaccharides and glycoprotein. βGlu I is transcriptionally induced in tobacco mosaic virus (TMV)-infected leaves as part of the local lesion response associated with the hypersensitive reaction (HR), and appears not to be induced systemically as part of the systemic acquired resistance (SAR) syndrome. The class II and III βGlu are induced both locally in TMV-infected leaves and systemically in non-infected leaves of the same plant and are markers for SAR. Systemic accumulation of salicylic acid (SA) is associated with SAR, and treatment of tobacco plants with SA strongly induces the class II and III βGlu, but not, or only weakly, the class I βGlu. Promoter analyses support these findings and detected differences between class I and II βGlu genes concerning responsive regions for ethylene, ABA, SA, TMV and other factors.

Nicotiana seeds as a model system for the hormonal regulation of dormancy, after-ripening and germination

Seed development is completed by a period of maturation when water content decreases, ABA and storage proteins accumulate, and desiccation tolerance and primary dormancy are established. In many plants, including *Nicotiana* species, endogenous ABA is involved in the induction and also in the maintenance of the dormant state (Hilhorst, 1995; Bewley, 1997b; Li and Foley, 1997; Grappin *et al.*, 2000; Koornneef *et al.*, 2002). The *Solanaceae* family can be divided into two large subgroups (Judd *et al.*, 1999): (1) The *Cestroideae* subgroup, e.g. *Nicotiana* and *Petunia*, is characterized by straight to slightly bent embryos and prismatic to subglobose seeds and, typically, by capsules; and (2) the *Solanoideae* subgroup, e.g. *Capsicum*, *Lycopersicon* and *Physalis*, is characterized by curved embryos and flattened, discoid seeds, and often by berries. Because tomato as a model system for *Solanoideae*-type seeds has been the main focus of several excellent reviews (e.g. Hilhorst, 1995; Bewley, 1997a; Hilhorst *et al.*, 1998) and because the two types of seeds differ in various aspects, I first want to summarize what is known about tobacco as a model system for *Cestroideae*-type seeds.

The onset of dormancy in *Nicotiana tabacum* is correlated with a peak in ABA content at approximately 15–20 d after pollination (DAP), and ABA declines rapidly during further seed maturation (Yamaguchi-Shinozaki *et al.*, 1990; Jiang *et al.*, 1996; Phillips *et al.*, 1997). Tobacco seeds harvested 25 DAP are dark brown, the embryos are mature and have white cotyledons, the products of maturation-specific genes have accumulated, dormancy has been established, desiccation tolerance has been acquired and the moisture content is low (Kincaid, 1935; Yamaguchi-Shinozaki *et al.*, 1990; Jakobsen *et al.*, 1994; Jiang *et al.*, 1996; Phillips *et al.*, 1997; Leubner-Metzger and Meins, 2000). Seed dormancy is not established in transgenic tobacco expressing an anti-ABA antibody that causes deficiency in free ABA, resulting in precocious germination, embryos with green cotyledons, reduced accumulation of storage proteins and desiccation intolerance (Phillips *et al.*, 1997). The wilty mutants *aba1* and *aba2* of *Nicotiana plumbaginifolia* have reduced ABA contents and

exhibit precocious germination and reduced primary dormancy (Marin *et al*., 1996; Frey *et al*., 1999; Grappin *et al*., 2000). ABA-deficiency of the *aba2* mutant is due to a mutation in the *ABA2* gene, encoding zeaxanthin epoxidase, a key step in ABA biosynthesis. Antisense- and sense-*ABA2* transformation of *N. plumbaginifolia* resulted in decreased and increased ABA biosynthesis and seed dormancy, respectively (Liotenberg *et al*., 1999). This study also suggested that, as in *Arabidopsis* and tomato (Hilhorst, 1995; Bewley, 1997b; Li and Foley, 1997), in *Nicotiana* species only ABA produced by the embryo itself, but not maternal ABA, is necessary to impose a lasting dormancy.

In the case of tobacco, the embryo in the mature seed is surrounded by 3–5 layers of rather thick-walled endosperm cells. The periphery of the endosperm is pressed against the thin testa, which consists of an outer layer of cutinized and lignified dead cells and a living inner parenchyma layer (Avery, 1933; Matzke *et al*., 1993). The maternal origin of this living cell layer interposed between the endosperm and the dead outer testa is suggested by gene promoter studies and by genetic ablation (Czakó *et al*., 1992; Matzke *et al*., 1993; Fobert *et al*., 1994). In contrast to the micropylar cap, typical for tomato or pepper seeds, the micropylar testa and endosperm of tobacco seeds are not organized in such a morphologically distinct, cap-like structure (Arcila and Mohapatra, 1983; Hilhorst *et al*., 1998).

Rupture of the testa and of the endosperm are distinct and temporally separate events during the germination of tobacco seeds (Figs 1 and 2; Arcila and Mohapatra, 1983; Leubner-Metzger *et al*., 1995; http://www.leubner.ch/). A visible distinction between testa and endosperm rupture appears to be a general phenomenon of *Cestroideae*-type seeds and is not found in *Solanoideae*-type seeds (Petruzzelli, Müller, Hermann and Leubner-Metzger, unpublished data). Tobacco testa rupture starts near the funiculus and spreads in random directions along the ridges on the testa. Progress of testa rupture is facilitated by channels underlying the ridges. When seeds reach the advanced testa rupture stage, the micropylar endosperm covering the radicle tip is exposed as a dome-shaped structure. Microscopic studies showed that storage reserves are degraded in the micropylar endosperm cells prior to protrusion by the radicle; and that the endospermic hole, which has a smooth outline and is always formed at the micropylar end of germinating tobacco seeds, results from tissue dissolution rather than from the pushing action of the protruding radicle (Arcila and Mohapatra, 1983; Leubner-Metzger *et al*., 1995). Similar observations are obvious for other endospermic species and are correlated with weakening of the micropylar endosperm covering the radicle tip, e.g. in lettuce (Dutta *et al*., 1994) and in members of the *Solanaceae*, including pepper (Watkins *et al*., 1985), *Datura* (Mella *et al*., 1994) and tomato (Hilhorst, 1995; Bewley, 1997a; Toorop *et al*., 2000). Further support for the finding that the seed-covering layers impose a physical constraint to radicle protrusion during the germination of solanaceous species comes from surgical experiments. Removal of the micropylar testa and the endosperm tissues permits radicle growth under conditions that inhibit germination of intact seeds of tobacco (Bihlmeier, 1927; Kincaid, 1935), tomato (Liptay and Schopfer, 1983; Hilhorst, 1995) and potato (Fischnich and Lübbert, 1955).

Dormancy can be released during after-ripening, i.e. a period of dry storage of freshly harvested, mature seeds (Bewley, 1997b; Li and Foley, 1997). A further decline in ABA content, decreased sensitivity to ABA and increased sensitivity to GA are involved in the after-ripening-mediated transition from the dormant to the non-dormant state of many species (Hilhorst, 1995; Li and Foley, 1997; Debeaujon and Koornneef, 2000; Grappin *et al*., 2000; Koornneef *et al*., 2002). The work of Grappin *et al*. (2000) demonstrated this for *N. plumbaginifolia* and showed, in addition, *de novo* ABA biosynthesis in imbibed freshly harvested (dormant), but not after-ripened (non-dormant) seeds. The after-ripening-mediated promotion of *N. tabacum* germination is due to the promotion of both testa and subsequent endosperm rupture (Leubner-Metzger and Meins, 2000; Leubner-Metzger, 2002). This result was obtained by comparing the testa and endosperm rupture kinetics during imbibition in continuous light of tobacco seeds in the fresh state, i.e. mature, dormant seeds sampled at approximately 40 DAP, and the after-ripened state, i.e. seeds after storage several months under dry and warm conditions. Addition of ABA to the medium during imbibition resembles maternal ABA during seed development and residual ABA in mature seeds. Imbibition of freshly harvested or after-ripened tobacco seeds in medium with 10 µM ABA greatly delays endosperm rupture (Fig. 1A) and results in the formation of a novel structure, consisting of the enlarging radicle with a sheath of greatly elongated endosperm tissue (Leubner-Metzger *et al*., 1995; Leubner-Metzger and Meins, 2000). ABA treatment does not affect the kinetics of testa rupture of fresh or after-ripened tobacco seeds, but the delay in endosperm rupture depends on the ABA concentration.

According to the revised hormone-balance hypothesis for seed dormancy proposed by Karssen and Laçka (Karssen and Laçka, 1986), ABA and GA act at different times and sites during the 'seed life'. ABA induces dormancy during maturation and GA plays a key role in the promotion of germination. Light is required for at least two aspects of tobacco seed germination. First, in photodormant tobacco

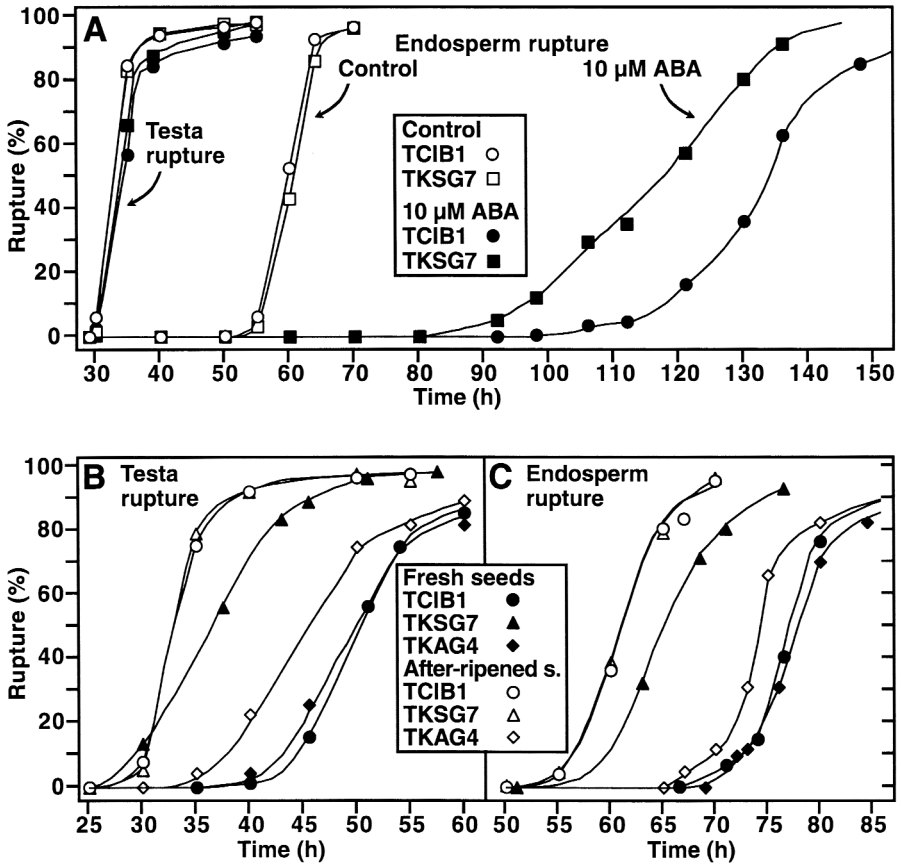

Figure 1. The effect of abscisic acid (ABA) (A), after-ripening (B, C) and class I β-1,3-glucanase (βGlu I) sense and antisense transformation on the kinetics of testa and endosperm rupture during tobacco seed germination. The incidence of testa and endosperm rupture is expressed as the percentage of sense-βGlu I (TKSG7), antisense-βGlu I (TKAG4) and empty-vector (TCIB1) seeds scored with time after the start of imbibition in continuous light. (A) The effect of ABA on testa and endosperm rupture of after-ripened seeds incubated in medium without (control) and with 10 µM ABA. (B, C) The effect of after-ripening on testa rupture (B) and endosperm rupture (C) investigated by comparing freshly harvested and after-ripened TKSG7, TKAG4 and TCIB1 seeds incubated in control medium. Note that βGlu I is overexpressed in TKSG7 seeds, but that TKAG4 seeds express TCIB1-like levels of βGlu I during endosperm rupture. The antisense-βGlu I transformation therefore only affects testa rupture of TKAG4 seeds, and the delay in endosperm rupture is attributed to the delay in testa rupture. Means of single-line mean values from several independent lines are presented; the single-line mean values ± SE are published in Leubner-Metzger and Meins (2000, 2001) and Leubner-Metzger (2002).

seeds, germination in darkness is blocked at a step before testa rupture, and neither testa nor endosperm rupture occur, even after several weeks of dark-imbibition (Kincaid, 1935; Kasperbauer, 1968; Mohapatra and Johnson, 1978; Leubner-Metzger *et al.*, 1996). Brief treatment of imbibed photodormant seeds with red light activates the phytochrome signal transduction pathway, resulting in the release of photodormancy and the promotion of germination (e.g. Kretsch *et al.*, 1995; Emmler and Schäfer, 1997). Genetic and physiological experiments suggest that tobacco photodormancy is mainly under maternal control (Bihlmeier, 1927; Honing, 1930; Kincaid, 1935; Kasperbauer, 1968; Leubner-Metzger, 2002). GA can substitute for the red-light trigger required to release photodormancy and to induce testa rupture and subsequent endosperm rupture of tobacco seeds imbibed in the dark (Leubner-Metzger *et al.*, 1996; Peng and Harberd, 2002). Red light up-regulates the biosynthesis of bioactive GA in germinating seeds of lettuce and *Arabidopsis* (Toyomasu *et al.*, 1998; Kamiya and Garcia-Martinez, 1999; Yamaguchi *et al.*, 2001). Far less is known about the role of GA sensitivity during the after-ripening-mediated release of photodormancy. Freshly harvested tobacco seeds are photodormant, and after-ripening contributes to the release of photodormancy (Kasperbauer, 1968; Leubner-Metzger and Meins, 2001; Leubner-Metzger, 2002). This effect varies greatly for different seed batches, as reported for several tobacco cultivars. The

GA requirements for photodormancy release of fresh and completely photodormant after-ripened seed batches are equal. Non-photodormant tobacco seeds have lost the requirement for exogenous GA for dark germination, which could be due to increased GA sensitivity and/or increased endogenous GA levels (Leubner-Metzger, 2002).

Light is also required for a second aspect of tobacco seed germination: it promotes the speed of endosperm rupture of non-photodormant tobacco seeds. GA is not only involved in inducing dark germination of photodormant tobacco seeds (Leubner-Metzger *et al.*, 1996; Leubner-Metzger, 2002), but it also promotes ABA-delayed endosperm rupture of dark-imbibed non-photodormant seeds (Leubner-Metzger, 2001), and osmoticum-delayed testa and endosperm rupture of light-imbibed seeds (Leubner-Metzger *et al.*, 1996). Promotion of ABA-delayed seed germination of *N. plumbaginifolia* by light or GA involves stimulation of ABA degradation and inhibition of ABA synthesis (Grappin *et al.*, 2000). Finally, endogenous ethylene and brassinosteroids also promote the germination of non-photodormant tobacco seeds and counteract the inhibitory effects of applied ABA on endosperm rupture, but ethylene and brassinosteroids do not release tobacco photodormancy (Leubner-Metzger *et al.*, 1998; Leubner-Metzger, 2001). Taken together, *Nicotiana* seeds provide an excellent model system for the investigation of germination and coat-imposed dormancy. We utilized the advantage that testa rupture and endosperm rupture are separate events to identify target sites for βGlu action.

βGlu I contributes to endosperm rupture

The first hint that βGlu may play a role in tobacco seed germination was our observation that βGlu I is induced during germination (Vögeli-Lange *et al.*, 1994; Leubner-Metzger *et al.*, 1995). Measurements of βGlu activity, βGlu I protein and mRNA content, in combination with reporter-gene experiments with the *Escherichia coli uidA* gene (*Gus* reporter-gene) fused to the promoters of the tobacco βGlu I genes *Gla* and *Glb*, established that most, if not all, of the βGlu activity is due to transcriptional induction of both βGlu I isoforms (Vögeli-Lange *et al.*, 1994; Leubner-Metzger *et al.*, 1995; Livne *et al.*, 1997). βGlu I is induced after testa rupture and just prior to endosperm rupture. This induction is localized exclusively in the micropylar endosperm at the site where the radicle will emerge. βGlu I induction during germination is not a classical defence-type response, since chitinases and the known acidic class II and III βGlu are not induced (Leubner-Metzger *et al.*, 1995; Leubner-Metzger and Meins, 1999). The 'PR-like' tobacco class IV βGlu *Tag1* gene is also not induced (G. Leubner Metzger, unpublished results). ABA inhibits βGlu I induction and endosperm rupture of germinating tobacco seeds in a dose-dependent manner. ABA also inhibits βGlu I induction and endosperm rupture of other *Cestroideae*-type seeds, including three other *Nicotiana* species and *Petunia hybrida* (Petruzzelli, Müller, Hermann and Leubner-Metzger, unpublished data). Class I βGlu mRNA, protein and enzyme activity are also expressed in the micropylar endosperm of tomato seeds prior to radicle protrusion (Wu *et al.*, 2000). As in tobacco, no accumulation of class II and III βGlu occurs, and the transcriptional induction of βGlu I, as well as germination, is inhibited by ABA. The βGlu I induction in the micropylar endosperm of tobacco (unpublished results) and tomato (Wu and Bradford, 2002) is not a wound response. In contrast to tobacco, class I chitinase also accumulates in germinating tomato seeds, but is not down-regulated by ABA and, in contrast to βGlu I, seems to be wound-induced (Wu *et al.*, 2000; Wu and Bradford, 2002). In addition, a different post-germinative tomato βGlu has been reported by Morohashi and Matsushima (2000). βGlu activity is also induced in other *Solanoideae*-type seeds, including *Capsicum annuum* and *Physalis peruvianum* (Petruzzelli, Müller, Hermann and Leubner-Metzger, unpublished data). As in tomato, ABA inhibits endosperm rupture and βGlu activity accumulation of pepper seeds, and ABA-sensitive βGlu accumulated in the micropylar cap prior to endosperm rupture. In contrast to tomato, where βGlu accumulation is confined to the micropylar endosperm, βGlu accumulation in pepper also occurred in other seed tissue. Also in contrast to tomato, chitinase did not accumulate in germinating pepper seeds (Wu *et al.*, 2000; Petruzzelli, Müller, Hermann and Leubner-Metzger, unpublished data). Thus, ABA-sensitive accumulation of βGlu in the micropylar endosperm prior to its rupture by the protruding radicle appears to be a widespread phenomenon during the germination of *Solanoideae*- and *Cestroideae*-type seeds, whereas chitinase accumulation appears to be a species-dependent feature.

The induction of βGlu I and endosperm rupture are tightly linked in response to physiological factors known to affect the incidence and timing of germination (Fig. 2; Leubner-Metzger and Meins, 1999). Tobacco seed germination is accompanied by ethylene evolution, and endogenous ethylene is required for the promotion of endosperm rupture and high-level βGlu I expression of light-imbibed seeds and of non-photodormant dark-imbibed seeds (Leubner-Metzger *et al.*, 1998). Ethylene does not affect the spatial and temporal pattern of βGlu I expression and does not break photodormancy or affect the kinetics of testa rupture. Regulation of βGlu I and class I chitinase is often tightly coordinated, e.g.

in leaves both accumulate in response to ethylene treatment and infection by pathogens (Leubner-Metzger and Meins, 1999). In contrast, ethylene induces βGlu I, but not class I chitinase, in germinating tobacco seeds, and ABA transcriptionally down-regulates βGlu I, but not class I chitinase in tobacco leaf tissue (Rezzonico *et al.*, 1998). ABA inhibits the induction of the βGlu I genes during tobacco seed germination and specifically delays endosperm rupture, but does not affect the kinetics of testa rupture (Fig. 1A; Leubner-Metzger *et al.*, 1995). While ABA delays the rate of βGlu I accumulation and the timing of endosperm rupture in a concentration-dependent manner, it does not affect the onset of βGlu I induction and does not confer a complete block to βGlu I accumulation or endosperm rupture. Kinetic analysis of the ABA effect in light-imbibed, after-ripened seeds strongly suggests that endosperm rupture depends on a critical threshold concentration of βGlu I. A similar effect has been found for osmoticum-imbibed tomato seeds, in which endo-β-mannanase accumulation, endosperm weakening and rupture depend on crossing a threshold water potential (Toorop *et al.*, 1998). βGlu I accumulation and endosperm rupture are also delayed in osmoticum-imbibed tobacco seeds (Leubner-Metzger *et al.*, 1996).

GA treatment can replace light in promoting βGlu I accumulation and endosperm rupture of non-photodormant tobacco seeds imbibed in the dark in medium without and with 10 μM ABA (Leubner-Metzger, 2001). Photodormant tobacco seeds imbibed in the dark do not germinate and do not accumulate βGlu I, but GA-mediated release of photodormancy induced βGlu I in the dark in association with endosperm rupture (Leubner-Metzger *et al.*, 1996). Seeds of the GA-deficient *gib-1* mutant of tomato do not accumulate βGlu I and do not germinate, but treatment with GA induces βGlu I gene expression in the micropylar endosperm, followed by germination (Wu *et al.*, 2000). A promoter deletion analysis of the tobacco βGlu I B (*Glb*) gene in germinating tobacco seeds (Leubner-Metzger *et al.*, 1998) suggests that the distal region, which contains the positively acting ethylene-responsive element (ERE), is required for high-level, ethylene-sensitive expression; that the proximal region is necessary and sufficient for low-level micropylar-endosperm specific expression; and that both regions contribute to down-regulation by ABA. These promoter regions contain several highly conserved *cis*-acting elements for the regulation by tissue-specific factors, GA, ABA and ethylene (Leubner-Metzger and Meins, 1999; Leubner-Metzger, 2001). Enhancer activity and ethylene responsiveness of βGlu I depend on the AGCCGCC box present as two copies in the ethylene-responsive element (ERE). They are the binding site of ERE binding proteins (EREBPs), which are transcription factors mediating ethylene responses (Ohta *et al.*, 2000). Transcripts of the EREBPs showed a germination- and hormone-specific expression pattern in tobacco seeds (Leubner-Metzger *et al.*, 1998).

The close correlation between βGlu I induction and the onset of endosperm rupture under a variety of physiological conditions supports the hypothesis that βGlu I contributes to endosperm rupture. Direct evidence for a causal role of βGlu I during endosperm rupture comes from sense-transformation with a chimeric ABA-inducible βGlu I transgene (Leubner-Metzger and Meins, 2000). Tobacco plants were transformed with a sense-βGlu I construct consisting of the genomic DNA fragment of the tobacco *βGlu I B* gene regulated by the castor bean *Cat1* gene promoter. The *Cat1* promoter is known to confer ABA-inducible, endosperm-specific transgene expression in germinating tobacco seeds (Suzuki *et al.*, 1995; Leubner-Metzger and Meins, 2000; Leubner-Metzger, 2002). Seeds were obtained from independent sense-βGlu I lines (TKSG7) and, for the purpose of proper controls, from independent empty-vector lines (TCIB1). Sense-βGlu I transformation results in overexpression of βGlu I mRNA, protein and activity in TKSG7 seeds and promotes endosperm rupture of fresh, mature seeds and ABA-treated after-ripened seeds (Fig. 1A; Leubner-Metzger and Meins, 2000). In contrast to fresh and to ABA-treated after-ripened TKSG7 seeds, βGlu I overexpression does not promote endosperm rupture of after-ripened TKSG7 seeds imbibed in medium without added ABA (Fig. 1A). This result supports our earlier finding that a critical threshold concentration of βGlu I is sufficient for proper germination under optimal conditions (Leubner-Metzger *et al.*, 1995). ABA down-regulates the βGlu I host genes in TCIB1 and wild-type seeds, but in the presence of the ABA-inducible βGlu I-transgene, ABA causes high-level βGlu I expression in TKSG7 seeds (Leubner-Metzger and Meins, 2000). ABA treatment delays endosperm rupture of after-ripened TCIB1 and TKSG7 seeds, but, due to βGlu I overexpression, this delay is significantly reduced in TKSG7 seeds (Fig. 1A). βGlu I overexpression reduces the ABA-mediated delay in endosperm rupture of fresh and after-ripened seeds, but ABA treatment does not affect the kinetics of testa rupture (Leubner-Metzger and Meins, 2000; Leubner-Metzger, 2002). In agreement with increased endogenous ABA content and signalling in fresh seeds (Grappin *et al.*, 2000), faster βGlu I accumulation and germination are obvious in fresh TKSG7 seeds imbibed in medium without ABA (Leubner-Metzger and Meins, 2000). Taken together, these results support the view that a threshold βGlu I content is required, but not sufficient, for endosperm rupture. In the presence of ABA, βGlu I becomes a limiting factor for endosperm

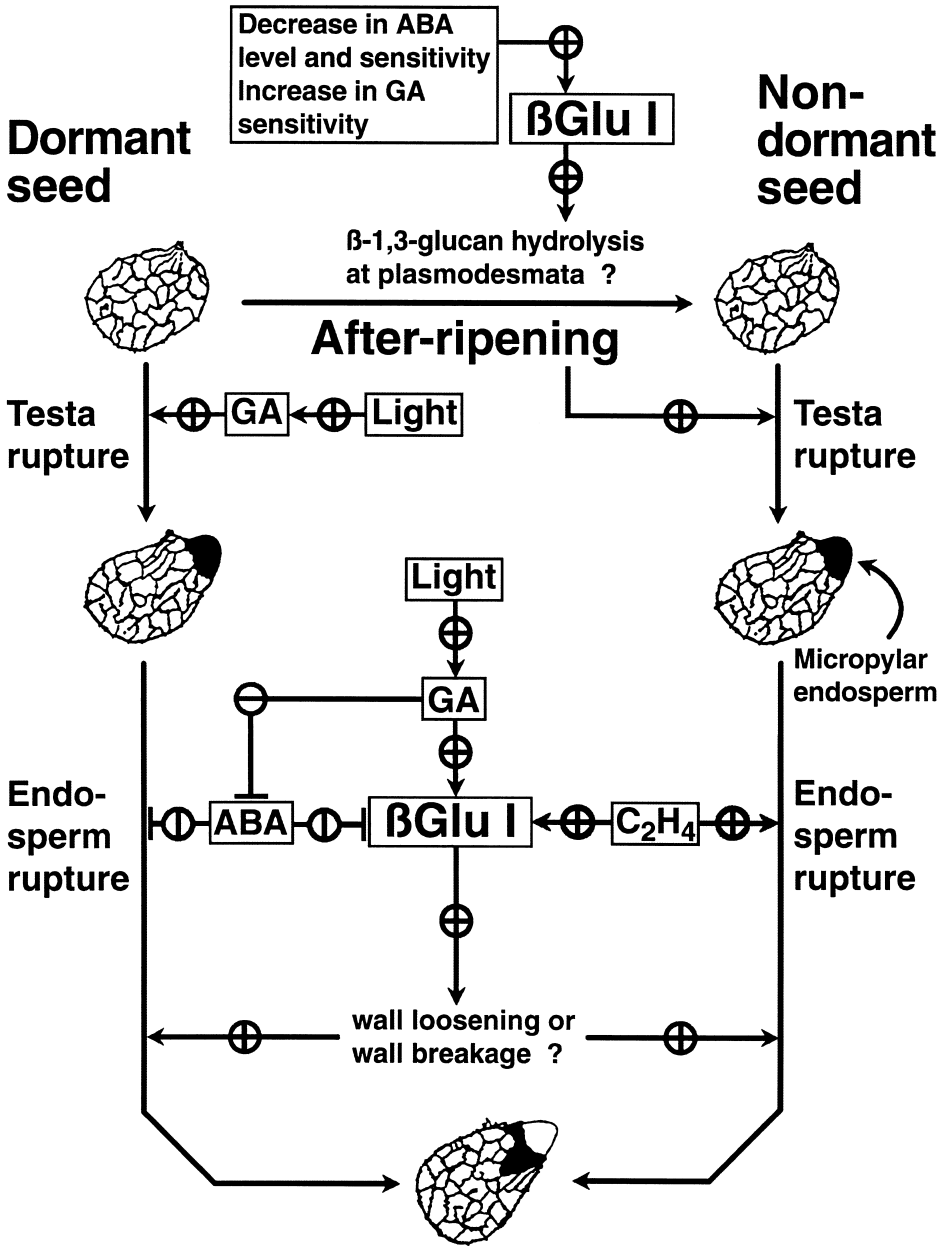

Figure 2. A speculative model integrating tobacco class I β-1,3-glucanase (βGlu I), seed dormancy, after-ripening and germination. According to the model, βGlu I expression is transcriptionally down-regulated by abscisic acid (ABA) in dormant seeds. Expression of βGlu I contributes to the release of coat-imposed dormancy and the promotion of germination, by acting at two sites. First, decrease in ABA level and sensitivity during after-ripening eventually permit βGlu I expression in seeds. Upon sufficient hydration, increased βGlu I action contributes to the release of coat-imposed dormancy and promotes testa rupture in the light. Secondly, βGlu I is induced by the light/gibberellin (GA) pathway in the micropylar endosperm and facilitates endosperm rupture. Endosperm-specific βGlu I expression and endosperm rupture are inhibited by ABA and promoted by light, GA and ethylene (C_2H_4). The light/GA pathway also promotes ABA degradation. In addition, photodormancy is manifested as a block prior to testa rupture during dark imbibition. It can either be released during after-ripening or by the light/GA pathway early during imbibition. βGlu I is one of several key factors that regulate dormancy and germination in response to environmental and hormonal conditions. A 'plus' sign designates promotion and a 'minus' sign inhibition of a process.

rupture (Fig. 2), and removal of this block, due to expression of the ABA-inducible βGlu I-transgene in TKSG7 seeds, promotes endosperm rupture until other ABA-sensitive processes become limiting. While these results do not show how βGlu I promotes endosperm rupture, they show directly that βGlu I is causally involved and that it contributes substantially to endosperm rupture.

Occurrence and functions of β-1,3-glucans in seeds

Little is known about the molecular mechanisms underlying the effects of βGlu on seed dormancy and germination. A possible function for βGlu in dicot stem elongation growth has been proposed (Simmons, 1994; Cosgrove, 1999), but this function is unproven. Although this is a possible hypothesis, there is no evidence demonstrating that βGlu expression in the micropylar endosperm acts by promoting the growth potential of the embryo. We proposed as a working hypothesis that βGlu contributes to degradation of cell-wall material, resulting in endosperm weakening and promotion of radicle protrusion, i.e. endosperm rupture (Vögeli-Lange *et al.*, 1994; Leubner-Metzger *et al.*, 1995). One possibility is that βGlu acts by digesting the β-1,3-glucan callose (Simmons, 1994; Leubner-Metzger and Meins, 1999), which is deposited between the plasma membrane and the cell wall of many tissues. Possible functions of callose in plants include: physical and chemical isolation of developing gametes, cell division, bud dormancy, protection from environmental and osmotic stress, regulation of plasmodesmatal trafficking, matrix for deposition of other wall components, pollen tube growth and plant–microbe interactions (e.g. Kelly *et al.*, 1992; Bucciaglia and Smith, 1994; Iglesias and Meins, 2000; Sivaguru *et al.*, 2000; Doblin *et al.*, 2001; Rinne *et al.*, 2001; Scherp *et al.*, 2001). Callose is present in large quantities in the seed-covering layers of several dicot species (e.g. Kelly *et al.*, 1992; Welbaum *et al.*, 1998; Wittich and Graven, 1998; Yim and Bradford, 1998; Nguyen *et al.*, 2002). In legume seeds it has been proposed to be the reason for water impermeability of the coats (Kelly *et al.*, 1992). Semipermeable layers, characterized by allowing water uptake and gas exchange while restricting solute diffusion, have been localized to the seed-covering structures of many species (e.g. Beresniewicz *et al.*, 1995; Yim and Bradford, 1998). In members of the *Cucurbitaceae* family (muskmelon, cucumber, zucchini, watermelon), seed semipermeability correlates with a thick aniline-blue-staining (indicative of callose) layer (Welbaum *et al.*, 1998; Yim and Bradford, 1998). In muskmelon, a single layer of endosperm cells, covered by a thick deposit of callose and a thin waxy or suberin- and lipid-containing outer layer, encloses the embryo and creates a semipermeable apoplastic envelope. When dead muskmelon seeds are allowed to imbibe, solutes leaking from the embryo are retained within the envelope, resulting in osmotic water uptake and swelling. Acquisition of semipermeability and callose deposition in this layer coincide during seed development and are associated with distinct aniline-blue-staining vesicles in the endosperm envelope cells. Artificial degradation of the callose layer by treatment of decoated muskmelon seeds with exo-β-1,3-glucanase from *Helix pomatia* causes loss of semipermeability. The callose layer is rapidly degraded upon imbibition of intact seeds, in correlation with the loss of semipermeability. These results demonstrate that massive deposition of callose in seed-covering layers can serve as a semipermeable 'molecular filter' that readily allows movement of water but not of solutes (Welbaum *et al.*, 1998; Yim and Bradford, 1998). Callose can not only serve as a 'molecular sieve', but also as a wall-strengthening agent, and it is proposed that an, as yet uncharacterized, β-glucanase in the muskmelon endosperm is related to weakening and radicle emergence (Welbaum *et al.*, 1998). As in muskmelon, semipermeability of barley grains also correlates with an aniline-blue-staining layer inside the seed coat (stated in Yim and Bradford, 1998). It is not known whether such a callose layer is a common feature of cereal grains, how the β-1,3-glucanases are regulated that might degrade it and whether this is connected to increased ABA leakage and termination of dormancy (e.g. Cordero *et al.*, 1994; Visser *et al.*, 1996; Benech-Arnold *et al.*, 1999).

Massive callose accumulation is not a general feature of semipermeable layers in seed-covering structures. Histochemical staining with aniline blue did not detect callose in seeds of tomato, pepper, lettuce, leek or onion (Beresniewicz *et al.*, 1995; Yim and Bradford, 1998). Purified βGlu I of tobacco and tomato causes no significant release of reducing sugars from crude cell walls, isolated from tomato micropylar endosperm (with the testa removed) (Wu *et al.*, 2000). Thus, there is no evidence for substrates of these endo-type βGlu in tomato micropylar endosperm cell walls. These findings do not exclude the presence of βGlu substrates in tomato seeds *per se.* Micropylar testa cell walls have not been tested for possible βGlu hydrolysis products, and callose is not the only possible substrate. βGlu can also act indirectly by releasing minute amounts of elicitor-active β-1,3-glucan oligosaccharides, and endo-type βGlu can exhibit transglycosylation activity, which is not detectable with this type of assay (Boller, 1995; Sova *et al.*, 1997; Klarzynski *et al.*, 2000). Endosperm weakening appears to be a prerequisite for tomato

germination, and is likely to be achieved by cell-wall hydrolysis by the collaborative or successive action of several phytochrome- and GA-regulated cell-wall hydrolases (Bewley, 1997a). Two phases can be distinguished: (1) The early phase is not inhibited by ABA and includes ABA-insensitive endosperm weakening associated with micropylar-endosperm specific, GA-inducible and ABA-independent expression of endo-β-mannanase, expansin and other proteins, but not βGlu I expression (e.g. Bewley, 1997a; Chen and Bradford, 2000; Nonogaki *et al.*, 2000; Toorop *et al.*, 2000; Wu *et al.*, 2000). Endo-β-mannanase, which can hydrolyse isolated micropylar endosperm cell walls *in vitro*, appears to be necessary for endosperm weakening, but is not sufficient for the completion of tomato germination. (2) The late phase is critical, since it includes the final ABA-controlled step of radicle emergence associated with ABA-sensitive βGlu I expression in the micropylar endosperm, and βGlu I could therefore contribute to radicle emergence of tomato (Wu *et al.*, 2000). It is proposed that the late phase includes a second, ABA-controlled step of endosperm weakening, which is a biphasic process in tomato (Toorop *et al.*, 2000). Tomato endosperm weakening is usually measured as the force required to puncture micropylar seed halves that include the endosperm plus the testa tissues (Chen and Bradford, 2000; Toorop *et al.*, 2000; Wu *et al.*, 2000). The micropylar endosperm confers the major part of the mechanical resistance (Groot and Karssen, 1987). The testa accounts for approximately 20% of the mechanical resistance during the early phase of seed imbibition, and this declines just prior to radicle protrusion. The importance of the micropylar testa in controlling the completion of tomato germination is also obvious from the experiments with the ABA-deficient *sit*^{w} mutant (Hilhorst and Downie, 1996). Thus, testa rupture could be important in the late phase and could be achieved by an ABA-sensitive process that is characterized by wall breakage at preformed breaking points. βGlu I could contribute to this process, but this remains to be demonstrated. Seed germination and coat-imposed dormancy of tobacco are also regulated by the testa and the endosperm (Leubner-Metzger, 2002). Due to its small size, puncture force measurements are not feasible with tobacco, and detection of callose in tobacco seeds is hampered by the high autofluorescence of the covering layers. Thus, as yet, there is no evidence for the presence of callose in solanaceous seeds or for massive amounts of a βGlu substrate in tomato endosperm cell walls.

In contrast to the massive accumulation of callose in some semipermeable seed layers, strategically localized callose deposition in the neck regions of plasmodesmata seems to be sufficient to regulate symplastic trafficking and bud dormancy (Iglesias and Meins, 2000; Sivaguru *et al.*, 2000; Rinne *et al.*, 2001). Plasmodesmata are intercellular connections that allow direct symplastic movement of water, nutrients, signalling molecules and macromolecules. Increased callose deposition is associated with decreased plasmodesmatal movement of fluorescence-labelled molecules, e.g. dyes of defined size, viruses, proteins and the plant hormone GA. β-1,3-Glucan synthetase and βGlu control the synthesis and degradation of callose, respectively. Increased callose deposition is associated with bud dormancy of trees, and release of bud dormancy by GA or chilling seems to involve callose degradation by βGlu (Krabel *et al.*, 1993; Rinne *et al.*, 2001). Rinne *et al.* (2001) have demonstrated that bud dormancy of birch involves callose deposition in the neck regions of plasmodesmata and, as a result, all symplastic transport of the meristem is shut down. Release of bud dormancy by chilling involves removal of the callose plugs from the plasmodesmata and regaining of the capacity for symplastic transport. Bud dormancy release seems to be mediated by βGlu present in small, spherosome-like vacuoles that arise *de novo* during dormancy induction. Chilling induces a shift of these βGlu-containing vacuoles towards the plasma membrane, which seems to be followed by βGlu release and action on plasmodesmatal callose. Removal of the callose deposits during the release of birch bud dormancy coincides with the production of a 32 kDa βGlu detected by a polyclonal anti-tobacco βGlu antibody that recognizes the class I (vacuolar) and class II (secreted) βGlu isoforms (Rinne *et al.*, 2001). Such a mechanism could also be involved in the release of seed dormancy by βGlu, but this remains to be demonstrated.

βGlu may also act by indirect release from cell walls of elicitor-active β-1,3-glucan oligosaccharides that may serve as signalling molecules for the induction or enhancement of processes related to endosperm weakening, cell death of seed layers, dormancy release or pathogen defence (Boller, 1995; Klarzynski *et al.*, 2000; Leubner-Metzger and Meins, 2001). The soybean elicitor-releasing βGlu are class III isoforms (e.g. Cheong *et al.*, 2000), and it is of particular interest that they share more than 60% amino-acid identity with the class III isoforms of tobacco and tomato. The known acidic class III βGlu of tobacco is not expressed in germinating tobacco seeds (Leubner-Metzger *et al.*, 1995), but the promoter of a soybean class III βGlu encoding a basic isoform is active in the micropylar endosperm of germinating tobacco seeds (Cheong *et al.*, 2000). Branched β-1,3;1,6-glucans and linear β-1,3-glucans (especially β-1,3-pentaglucan) are able to elicit defence-type responses in leaf tissue of tobacco, tomato and other species (Boller, 1995; Klarzynski *et al.*, 2000). These responses include the production of H_2O_2 and the

accumulation of PR proteins, but not the entire set of responses associated with programmed cell death. Minute quantities of β-1,3-glucan elicitors released from the cell walls of plants and/or fungal pathogens are sufficient to induce these responses. Whether β-1,3-glucan oligosaccharide elicitors are released in seeds and affect germination or seed-specific defence responses is not known, but, besides PR protein accumulation, some of the other responses have been reported. Reactive oxygen species (ROS; including $O^{\bullet}_{2}^{-}$, H_2O_2, $^{\bullet}OH$) are released by germinating seeds, and ROS production is under hormonal and developmental control (Schopfer *et al.*, 2001). ROS may cause cell-wall loosening, extension growth and promote germination (Amaya *et al.*, 1999; Schopfer, 2001; Schopfer *et al.*, 2001; Morohashi, 2002). ROS are implicated in endosperm development and in aleurone layer senescence during germination of cereals; and these processes are promoted by GA and ethylene and inhibited by ABA (e.g. Young and Gallie, 2000; Bethke and Jones, 2001). The endosperm of castor bean (Schmid *et al.*, 1999) and tomato (Lehmann *et al.*, 2001) exhibits programmed cell death as part of the post-germinative mobilization of storage reserves. This is a very interesting area for future experiments, but to date there is no direct proof for a role of programmed cell death in the micropylar endosperm of germinating dicot seeds.

In summary, endosperm rupture of seeds with endosperm-limited germination appears to be a complex process. There is strong evidence that major endosperm weakening by the action of endo-β-mannanase is necessary for tomato endosperm rupture, and this appears to be achieved by direct digestion of a polymeric β-1,4-mannose substrate located in the cell walls (Groot *et al.*, 1988; Bewley, 1997a; Nonogaki *et al.*, 2000; Toorop *et al.*, 2000; Wu *et al.*, 2000). Direct proof for an *in vivo* role of endo-β-mannanase by sense- and antisense-transformation of tomato is still lacking. Although it appears to be necessary, endo-β-mannanase-mediated endosperm weakening alone is not sufficient for the completion of tomato germination. ABA clearly controls the final step of endosperm rupture and neither endo-β-mannanase accumulation nor the weakening caused by it are inhibited by ABA. A possible second step of ABA-sensitive endosperm weakening has been proposed for tomato (Toorop *et al.*, 2000). ABA-sensitive βGlu might contribute to this second step, but there is yet no evidence for a β-1,3-glucan substrate in the walls of tomato endosperm cells (Wu *et al.*, 2000). The hormonal regulation of βGlu expression by ABA, GA and ethylene is consistent with a role of this enzyme in the endosperm rupture of solanaceous species (Leubner-Metzger *et al.*, 1998; Wu *et al.*, 2000; Petruzzelli, Müller, Hermann and Leubner-Metzger, unpublished data). Direct evidence obtained by sense-βGlu I transformation demonstrates that ABA-inhibitable βGlu I contributes substantially to the endosperm rupture of tobacco (Leubner-Metzger and Meins, 2000). These findings show directly that expression of βGlu I in the endosperm is causally involved in promoting endosperm rupture of tobacco, and are consistent with the hypothesis of Ikuma and Thimann (1963), but a β-1,3-glucan substrate has not yet been found in tobacco seeds. Other cell-wall modifying proteins and modes of action might be involved in endosperm weakening (e.g. Bewley, 1997a; Chen *et al.*, 2001; Morohashi, 2002). Taken together, endosperm weakening appears to be achieved by the concerted action of several proteins, and by the sum of distinct mechanisms affecting the micropylar endosperm cell walls. There appear to be hormonal, environmental and species-specific differences in the regulation of these subprocesses.

Effects of βGlu I and after-ripening on testa rupture and photodormancy

Sense and antisense transformation provided more indirect evidence for a second, novel site of βGlu I action on the release of dormancy during tobacco seed after-ripening (Leubner-Metzger and Meins, 2000, 2001). Since βGlu I expression is not inhibited in the antisense seeds during endosperm rupture, no conclusion can be drawn from the antisense approach about the effect of βGlu I on endosperm rupture. However, testa rupture of after-ripened antisense-βGlu I seeds in the light is delayed compared to that of after-ripened, wild-type, TCIB1 and TKSG7 seeds (Leubner-Metzger and Meins, 2000, 2001; Fig. 1B). After-ripening promotes the germination of light-imbibed tobacco wild-type and TCIB1 seeds, and a recent study (Leubner-Metzger, 2002) has shown that after-ripening causes an earlier onset of testa rupture, followed by a similarly earlier onset of endosperm rupture (Fig. 1B, C). In contrast with after-ripened seeds, germination in the light is not affected in fresh antisense-βGlu I seeds, but is promoted in fresh sense-βGlu I seeds (TKSG7). Thus, not only can the promoting effect of after-ripening on germination be replaced by βGlu I overexpression, but the effect of both factors can be detected at the stage of testa rupture. Reciprocal genetic crosses between wild-type tobacco and homozygous, monogenic TKSG7 lines show that βGlu I is overexpressed in the covering layers and may replace the promoting effect of after-ripening on testa rupture only if the mother plant is a TKSG7 line. Although an effect on testa rupture of high-level βGlu I expression in the endosperm is also possible, it seems more likely that βGlu I expression in the maternal TKSG7-derived testa tissue is

necessary and sufficient for conferring the promoting effects of after-ripening on the onset of testa rupture. Germination of tomato is also controlled by ABA, and both micropylar covering layers contribute to coat-imposed dormancy (Groot and Karssen, 1987; Hilhorst, 1995; Wu *et al.*, 2000). Increased βGlu I expression (Leubner-Metzger, unpublished results) and a thinner micropylar testa are correlated with faster germination of the ABA-deficient *sit*^{w} tomato mutant (Hilhorst and Downie, 1996). These authors conclude from their experiments that both the micropylar testa and the micropylar endosperm are important in controlling the completion of germination, i.e. radicle emergence. Relationships among ABA, testa characteristics, coat-imposed dormancy and its release during after-ripening seem to be common features of endospermic and non-endospermic seeds (e.g. Kelly *et al.*, 1992; Hilhorst and Downie, 1996; Welbaum *et al.*, 1998; Debeaujon and Koornneef, 2000; Debeaujon *et al.*, 2000; Koornneef *et al.*, 2002; Leubner-Metzger, 2002).

Sense-βGlu I transformation of tobacco has no detectable effects on photodormancy of fresh seed, on the after-ripening-mediated release of photodormancy, or on the GA requirement for photodormancy release (Kasperbauer, 1968; Leubner-Metzger and Meins, 2001; Leubner-Metzger, 2002). The finding that photodormancy release during after-ripening is inhibited in antisense-βGlu I seeds suggests that βGlu I is necessary, but not sufficient, for the complete transition to non-photodormancy. In general, modulation of dormancy during after-ripening results in a broadening of the germination responses to environmental conditions (Bewley, 1997b; Li and Foley, 1997; Koornneef *et al.*, 2002). βGlu I is one of the factors regulating the release of tobacco coat-imposed seed dormancy and has at least two target sites (Fig. 2): (1) Testa rupture, which is not affected by ABA during imbibition, but is promoted by after-ripening and by βGlu I overexpression in the covering layers, and (2) endosperm rupture, which is inhibited by ABA during imbibition, and depends on the contribution of ABA-sensitive expression of βGlu I during germination.

β-1,3-Glucanases during the germination of non-endospermic dicot seeds

In non-endospermic species, endosperm assimilation occurs during seed development. A developmentally regulated βGlu is expressed during pea seed development in the immature endosperm and testa layers (Buchner *et al.*, 2002). In many mature non-endospermic seeds, the cotyledons are the sole storage organs and the embryo is enclosed by the testa as the sole covering layer (e.g. Schopfer and Plachy, 1984, 1993; Kretsch *et al.*, 1995). The testa produces a restraint during germination of radish (Schopfer and Plachy, 1993) and *Arabidopsis* (Debeaujon and Koornneef, 2000; Debeaujon *et al.*, 2000), but the testa is no hindrance during germination of rape (Schopfer and Plachy, 1984) and pea (Petruzzelli *et al.*, 2000). Ethylene promotes ethylene biosynthesis during the germination of non-dormant pea seeds by positive feedback regulation of 1-aminocyclopropane-1-carboxylic acid oxidase, and radicle protrusion through the testa is accompanied by an increase in ethylene evolution (Petruzzelli *et al.*, 1999, 2000). βGlu and chitinase show novel tissue-specific patterns of ethylene-dependent and ethylene-independent regulation during pea germination. βGlu activity levels, ethylene-responsiveness and biosynthesis remain low in cotyledon tissue. Ethylene responsiveness and biosynthesis increase in the embryonic axis during the late phase of pea germination. A strong increase in βGlu activity in the embryonic axis just after the completion of radicle emergence depends on ethylene and is due to a 34.5 kDa βGlu. High constitutive levels of chitinase are present in cotyledons and the embryonic axis. Thus, after the completion of radicle emergence, when βGlu is induced in the embryonic axis, antifungal combinations of the enzymes are present and might constitute a pre-emptive strategy to protect germinating pea seeds against microbial attack. βGlu isolated from cowpea seeds are antifungal against seed pathogens *in vitro* (Gomes *et al.*, 1996). In addition, a possible function for βGlu in dicot stem elongation growth has been proposed (Simmons, 1994; Cosgrove, 1999).

β-1,3-Glucanases during the germination of cereal caryopses

1,3;1,4-β-Glucans are the predominant non-cellulosic polysaccharides in the cell walls of grasses and, as a major component of the matrix hemicelluloses, function in binding cellulose and cross-linking the microfibrils (Høj and Fincher, 1995; Cosgrove, 1999; Hrmova and Fincher, 2001). They are not found in dicots, and xyloglucan may serve a similar cross-linking role in dicot cell walls. 1,3;1,4-β-Glucans are especially abundant in the starchy endosperm of cereal grains, where they account for up to 75% of the wall polysaccharides. 1,3;1,4-β-Glucanases, which are not known in dicots, have been thoroughly characterized in grasses, and their function in the starchy endosperm of germinated grains is quite clear. They hydrolyse the 1,3;1,4-β-glucans and provide access for other hydrolytic enzymes during the post-germination mobilization of storage reserves. Less is known about the function of cereal endo-type βGlu,

which are present in the ungerminated grain and rise markedly during germination (Simmons, 1994; Høj and Fincher, 1995; Hrmova and Fincher, 2001). βGlu transcripts are preferentially expressed in the maternal tissues of developing barley caryopses (Sreenivasulu *et al*., 2002). Callose, a β-1,3-glucan substrate, is present in the nucellar projection and the vascular tissue of developing cereal grains, and might have a role in regulating plasmodesmatal transport of assimilates and/or protecting the developing endosperm from enzymes that break down the nucellar projection (Asthir *et al*., 2001). Semipermeability of mature cereal caryopses correlates with a callose layer on the inside of the seed coat (Yim and Bradford, 1998). In addition, callose is detected as small deposits throughout the starchy endosperm and the aleurone layer, where it seems to be associated with plasmodesmata (Meikle *et al*., 1994; Høj and Fincher, 1995; Brown *et al*., 1997). The cereal endo-type βGlu and 1,3;1,4-β-glucanases differ in substrate specificity, in that 1,3;1,4-β-glucanases do not hydrolyse β-1,3-glucans and vice versa. In dry, mature caryopses of barley, endo-type βGlu activity is found to be associated predominantly with the embryo (in particular the scutellum); this activity increases markedly in the aleurone layer and the starchy endosperm during germination, and GA treatment of whole grains or isolated aleurone layers enhances its secretion (Simmons, 1994; Høj and Fincher, 1995; Hrmova and Fincher, 2001). Barley endo-type βGlu constitute a small gene family with seven known members (GI to GVII) encoding intracellular and apoplastic isoforms (Simmons, 1994; Høj and Fincher, 1995). The high levels of βGlu suggest a pre-emptive strategy to protect the grain against microbial attack, which will be discussed later in this review. It has also been proposed that the endo-type βGlu participate in the initial release of β-glucans from endosperm walls (Bathgate *et al*., 1974), but direct evidence for such a role is not available. The differential regulation of βGlu and chitinases expressed in *Fusarium*-infected maize and wheat kernels suggests that some isoforms have unknown developmental functions during germination (Cordero *et al*., 1994; Caruso *et al*., 1999).

In addition to endo-type βGlu and 1,3;1,4-β-glucanases, 'broad-specificity' exo-β-glucanases have been characterized in grasses (e.g. Kim *et al*., 2000; Harvey *et al*., 2001; Hrmova and Fincher, 2001; Hrmova *et al*., 2002), and a few reports suggest that homologues also exist in dicots. Exo-β-glucanases are known to hydrolyse 1,3;1,4-β-glucans, 1,3-β-glucans, xyloglucans, a range of other β-glucans and β-oligoglucosides with (1,2)-, (1,4)-, and (1,6)-β-D-linkages. In addition, transferase activity has been reported (Kim *et al*, 2000; Hrmova *et al*., 2002). A role in wall loosening during coleoptile extension growth has been proposed, but compelling evidence for this hypothesis is still lacking (Kim *et al*., 2000; Hrmova and Fincher, 2001). *ExoI* and *ExoII* are two barley genes encoding exo-β-glucanases (Harvey *et al*., 2001). Only *ExoI* mRNA is present at low abundance in the GA-treated aleurone layer, both genes are transcribed in the scutellum, but only *ExoII* mRNA is found in germinated grains. It is proposed that exo-β-glucanases contribute to 1,3;1,4-β-glucan turnover during scutellum senescence. Another proposal is that exo-β-glucanases might act in concert with endo-type βGlu to degrade walls of invading fungi (Hrmova and Fincher, 2001). β-Glucosidases, acting on specific oligoglucosides and in some cases also on β-1,3-glucans, are also present in caryopses (e.g. Hasegawa *et al*., 1994; Fieldes and Gerhardt, 2001). Finally, two thaumatin-like proteins with β-1,3-glucan binding activity have been purified from germinated barley grain (Grenier *et al*., 1999; Osmond *et al*., 2001).

Seed pathogen-related functions of β-1,3-glucanase

There is compelling evidence that βGlu and chitinases, acting alone and particularly in combination, can help defend plants against fungal infection (reviews: Simmons, 1994; Leubner-Metzger and Meins, 1999; Gomez *et al*., 2002). These glucanohydrolases seem to act in two different ways: directly, by degrading the cell walls of the pathogen and, indirectly, by promoting the release of cell-wall-derived materials that can act as elicitors of defence reactions. The intracellular class I βGlu and chitinase isoforms of tobacco and tomato, but not of the apoplastic class II isoforms, act against some pathogens *in vitro*, but are without effect against others (for references see Leubner-Metzger and Meins, 1999). Similarly, only in some cases did high-level expression of class I or II βGlu and chitinases in transgenic plants result in increased resistance against a particular fungus. Taken together, the general conclusion is that it depends on the particular plant–fungus interaction whether or not a particular βGlu is involved in plant defence. This finding is also of utmost importance for investigating the hypothesis that βGlu and chitinases are part of a pre-emptive strategy to protect a germinating seed against microbial attack. This hypothesis was first proposed for germinating cereal grains (review: Høj and Fincher, 1995) and later for germinating seeds of dicot species (e.g. Gomes *et al*., 1996; Petruzzelli *et al*., 1999; Wu *et al*., 2000). In favour of this hypothesis, several of the βGlu isoforms isolated from germinating cereal grains act *in vitro* against grain pathogens (e.g. Leah *et al*., 1991; Seetharaman *et al*., 1997). The distinct regulation of βGlu and chitinase isoforms in

Fusarium-infected maize and wheat kernels suggests that some function in pathogen defence, while others have developmental functions during germination (Cordero *et al.*, 1994; Caruso *et al.*, 1999). Simultaneous expression of *in vitro* antifungal βGlu and chitinase in tomato, cowpea and pea also suggests a function in pathogen defence (Gomes *et al.*, 1996; Petruzzelli *et al.*, 1999; Wu *et al.*, 2000). In addition, some thaumatin-like proteins isolated from germinating barley grains bind β-1,3-glucans and inhibit the growth of certain fungal species *in vitro*, but are without effect against others (Grenier *et al.*, 1999; Osmond *et al.*, 2001). Thus, there is strong *in vitro* evidence supporting the view that certain βGlu isoforms are involved in certain seed–pathogen interactions, but *in vivo* evidence is lacking and offers an interesting research area for seed biology. Finally, pathogen-related and developmental roles are not mutually exclusive, and a certain βGlu could serve functions in seed defence as well as in germination.

A speculative model for tobacco seed dormancy and germination

A speculative model integrating tobacco seed dormancy, after-ripening, βGlu I and germination is presented in Fig. 2. According to the model, βGlu I, which is transcriptionally down-regulated by ABA, contributes to the release of coat-imposed dormancy and the promotion of germination at two sites. The first site of βGlu I action affects the after-ripening-mediated promotion of testa rupture of seeds imbibed in the light. Decreasing ABA content and sensitivity during after-ripening eventually permit βGlu I expression in seeds. Upon imbibition this βGlu I possibly hydrolyses plasmodesmatal β-1,3-glucan deposits and intensifies processes improving germination vigour. This may result in enhanced symplastic communication, e.g. GA signalling, and in enhanced capacity for water imbibition. These are characteristic features associated with the transition from the dormant (fresh) to the non-dormant (after-ripened) state. A major part of this β-1,3-glucan is probably localized at the living inner parenchyma layer of the testa and the endosperm/testa border, and represents a maternal component conferring coat-imposed dormancy. Hydrolysis of this β-1,3-glucan results in the after-ripening-promoted testa rupture. Hydrolysis of plasmodesmatal β-1,3-glucan probably also facilitates symplastic movement of GA (or GA precursors) needed for the release of photodormancy by the GA/light signal transduction pathway. The second site of βGlu I action is during the final ABA-controlled phase of endosperm rupture (Fig. 2). ABA-sensitive expression of βGlu I in the micropylar endosperm contributes to radicle protrusion, and a threshold content of βGlu I is required and sufficient for this step. βGlu I could act directly by hydrolysis of endosperm wall components, resulting in the promotion of endosperm weakening and its rupture by the protruding radicle. βGlu I could also act indirectly by releasing elicitor-active oligo-β-1,3-glucans from plant wall components that act as signalling molecules to mediate loosening or breakage of the micropylar endosperm, e.g. by reactive oxygen species and/or programmed cell death. These processes, as well as βGlu I expression and endosperm rupture, are promoted by GA and ethylene and are inhibited by ABA. Therefore, βGlu I appears to be a key factor in regulating dormancy and germination in response to environmental and hormonal conditions.

Acknowledgements

I am grateful to Peter Schopfer (Institut für Biologie II, Botanik, Universität Freiburg, Germany), Peter Toorop (Royal Botanic Gardens, Kew, United Kingdom) and Luciana Petruzzelli (Istituto Biosintesi Vegetali, C.N.R., Milano, Italy) for their suggestions and critical comments. My research is supported by a grant from the Deutsche Forschungsgemeinschaft (LE 720/3), which is gratefully acknowledged.

References

Amaya, I., Botella, M.A., de la Calle, M., Medina, M.I., Heredia, A., Bressan, R.A., Hasegawa, P.M., Quesada, M.A. and Valpuesta, V. (1999) Improved germination under osmotic stress of tobacco plants overexpressing a cell wall peroxidase. *FEBS Letters* **457**, 80–84.

Arcila, J. and Mohapatra, S.C. (1983) Development of tobacco seedling. 2. Morphogenesis during radicle protrusion. *Tobacco Science* **27**, 35–40.

Asthir, B., Spoor, W., Duffus, C. and Parton, R.M. (2001) The location of (1-3)-β-glucan in the nucellar projection and in the vascular tissue of the crease in developing barley grain using a (1-3)-β-glucan-specific monoclonal antibody. *Planta* **214**, 85–88.

Avery, G.S.J. (1933) Structure and germination of tobacco seed and the developmental anatomy of the seedling plant. *American Journal of Botany* **20**, 309–327.

Bathgate, G.N., Palmer, G.H. and Wilson, G. (1974) The action of endo-β-1,3-glucanases on barley and malt β-glucans. *Journal of the Institute of Brewing* **80**, 278–285.

Benech-Arnold, R.L., Giallorenzi, M.C., Frank, J. and Rodriguez, V. (1999) Termination of hull-imposed dormancy in developing barley grains is correlated with changes in embryonic ABA levels and sensitivity. *Seed Science Research* **9**, 39–47.

Beresniewicz, M.M., Taylor, A.G., Goffinet, M.C. and Koeller, W.D. (1995) Chemical nature of a semipermeable layer in seed coats of leek, onion

(Liliaceae), tomato and pepper (Solanaceae). *Seed Science and Technology* **23**, 135–145.

Bethke, P.C. and Jones, R.L. (2001) Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. *The Plant Journal* **25**, 19–29.

Bewley, J.D. (1997a) Breaking down the walls – a role for endo-β-mannanase in release from seed dormancy? *Trends in Plant Science* **2**, 464–469.

Bewley, J.D. (1997b) Seed germination and dormancy. *The Plant Cell* **9**, 1055–1066.

Bihlmeier, M. (1927) Der Einfluss der Vorquellung und der Samenschale auf die Keimung lichtgeförderter Samen. *Jahrbücher für Wissenschaftliche Botanik* **67**, 702–732.

Boller, T. (1995) Chemoperception of microbial signals in plant cells. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 189–214.

Brown, R.C., Lemmon, B.E., Stone, B.A. and Olsen, O.A. (1997) Cell wall (1→3)- and (1→3, 1→4)-β-glucans during early grain development in rice (*Oryza sativa* L.). *Planta* **202**, 414–426.

Bucciaglia, P.A. and Smith, A.G. (1994) Cloning and characterization of *Tag1*, a tobacco anther β-1,3-glucanase expressed during tetrad dissolution. *Plant Molecular Biology* **24**, 903–914.

Buchner, P., Rochat, C., Wuilleme, S. and Boutin, J.P. (2002) Characterization of a tissue-specific and developmentally regulated β-1,3-glucanase gene in pea. *Plant Molecular Biology* **49**, 171–186.

Caruso, C., Chilosi, G., Caporale, C., Leonardi, L., Bertini, L., Magro, P. and Buonocore, V. (1999) Induction of pathogenesis-related proteins in germinating wheat seeds infected with *Fusarium culmorum*. *Plant Science* **140**, 87–97.

Chen, F. and Bradford, K.J. (2000) Expression of an expansin is associated with endosperm weakening during tomato seed germination. *Plant Physiology* **124**, 1265–1274.

Chen, F., Dahal, P. and Bradford, K.J. (2001) Two tomato expansin genes show divergent expression and localization in embryos during seed development and germination. *Plant Physiology* **127**, 928–936.

Cheong, Y.H., Kim, C.Y., Chun, H.J., Moon, B.C., Park, H.C., Kim, J.K., Lee, S.H., Han, C.D., Lee, S.Y. and Cho, M.J. (2000) Molecular cloning of a soybean class III β-1,3-glucanase gene that is regulated both developmentally and in response to pathogen infection. *Plant Science* **154**, 71–81.

Cordero, M.J., Raventos, D. and San Segundo, B. (1994) Differential expression and induction of chitinases and β-1,3-glucanases in response to fungal infection during germination of maize seeds. *Molecular Plant–Microbe Interactions* **7**, 23–31.

Cosgrove, D.J. (1999) Enzymes and other agents that enhance cell wall extensibility. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 391–417.

Czakó, M., Jang, J.-C., Herr, J.M. and Márton, L. (1992) Differential manifestation of seed mortality induced by seed-specific expression of the gene for diphtheria toxin A chain in *Arabidopsis* and tobacco. *Molecular and General Genetics* **235**, 33–40.

Debeaujon, I. and Koornneef, M. (2000) Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology* **122**, 415–424.

Debeaujon, I., Léon-Kloosterziel, K.M. and Koornneef, M. (2000) Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiology* **122**, 403–413.

Demolder, J., De Backer, M., Fiers, W. and Contreras, R. (1993) Phenotypic effects in *Saccharomyces cerevisiae* after regulated expression of β-1,3-glucanase from *Nicotiana plumbaginifolia*. *Journal of Biotechnology* **27**, 295–305.

Doblin, M.S., DeMelis, L., Newbigin, E., Bacic, A. and Read, S.M. (2001) Pollen tubes of *Nicotiana alata* express two genes from different β-glucan synthase families. *Plant Physiology* **125**, 2040–2052.

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

Emmler, K. and Schäfer, E. (1997) Maternal effect on embryogenesis in tobacco overexpressing rice phytochrome A. *Botanica Acta* **110**, 1–8.

Fieldes, M.A. and Gerhardt, K.E. (2001) Developmental and genetic regulation of β-glucosidase (linamarase) activity in flax seedlings. *Journal of Plant Physiology* **158**, 977–989.

Fischnich, O. and Lübbert, G. (1955) Fruchtbildung bei Kartoffeln und Förderung der Keimschnelligkeit ihrer Samen. *Beiträge zur Biologie der Pflanzen* **31**, 179–206.

Fobert, P.R., Labbé, H., Cosmopoulos, J., Gottlob-McHugh, S., Ouellet, T., Hattori, J., Sunohara, G., Iyer, V.N. and Miki, B.L. (1994) T-DNA tagging of a seed coat-specific cryptic promoter in tobacco. *The Plant Journal* **6**, 567–577.

Frey, A., Audran, C., Marin, E., Sotta, B. and Marion-Poll, A. (1999) Engineering seed dormancy by the modification of zeaxanthin epoxidase gene expression. *Plant Molecular Biology* **39**, 1267–1274.

Gomes, V.M., Oliveira, A.E.A. and Xavier-Filho, J. (1996) A chitinase and a β-1,3-glucanase isolated from the seeds of cowpea (*Vigna unguiculata* L. Walp) inhibit the growth of fungi and insect pests of the seed. *Journal of the Science of Food and Agriculture* **72**, 86–90.

Gomez, L., Allona, I., Casado, R. and Aragoncillo, C. (2002) Seed chitinases. *Seed Science Research* **12**, 217–230.

Grappin, P., Bouinot, D., Sotta, B., Miginiac, E. and Jullien, M. (2000) Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* **210**, 279–285.

Grenier, J., Potvin, C., Trudel, J. and Asselin, A. (1999) Some thaumatin-like proteins hydrolyse polymeric β-1,3-glucans. *The Plant Journal* **19**, 473–480.

Groot, S.P.C. and Karssen, C.M. (1987) Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutants. *Planta* **171**, 525–531.

Groot, S.P.C., Kieliszewska-Rokicka, B., Vermeer, E. and Karssen, C.M. (1988) Gibberellin-induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle protrusion. *Planta* **174**, 500–504.

Harvey, A.J., Hrmova, M. and Fincher, G.B. (2001) Regulation of genes encoding β-D-glucan glucohydrolases in barley (*Hordeum vulgare*). *Physiologia Plantarum* **113**, 108–120.

Hasegawa, R., Tada, T., Torii, Y. and Esashi, Y. (1994) Presence of β-cyanoalanine synthase in unimbibed dry seeds and its activation by ethylene during pre-germination. *Physiologia Plantarum* **91**, 141–146.

Helleboid, S., Chapman, A., Hendriks, T., Inze, D., Vasseur, J. and Hilbert, J.L. (2000) Cloning of β-1,3-glucanases expressed during *Cichorium* somatic embryogenesis. *Plant Molecular Biology* **42**, 377–386.

Hilhorst, H.W.M. (1995) A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research* **5**, 61–73.

Hilhorst, H.W.M. and Downie, B. (1996) Primary dormancy in tomato (*Lycopersicon esculentum* cv. Moneymaker): Studies with the *sitiens* mutant. *Journal of Experimental Botany* **47**, 89–97.

Hilhorst, H.W.M., Groot, S.P.C. and Bino, R.J. (1998) The tomato seed as a model system to study seed development and germination. *Acta Botanica Neerlandica* **47**, 169–183.

Høj, P.B. and Fincher, G.B. (1995) Molecular evolution of plant β-glucan endohydrolases. *The Plant Journal* **7**, 367–379.

Honing, J.A. (1930) Nucleus and plasma in the heredity of the need of light for germination in *Nicotiana* seeds. *Genetica* **12**, 441–476.

Hrmova, M. and Fincher, G.B. (2001) Structure–function relationships of β-D-glucan endo- and exohydrolases from higher plants. *Plant Molecular Biology* **47**, 73–91.

Hrmova, M., De Gori, R., Smith, B.J., Fairweather, J.K., Driguez, H., Varghese, J.N. and Fincher, G.B. (2002) Structural basis for broad substrate specificity in higher plant β-D-glucan glucohydrolases. *The Plant Cell* **14**, 1033–1052.

Iglesias, V.A. and Meins, F. (2000) Movement of plant viruses is delayed in a β-1,3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. *The Plant Journal* **21**, 157–166.

Ikuma, H. and Thimann, K.V. (1963) The role of the seed-coats in germination of photosensitive lettuce seeds. *Plant and Cell Physiology* **4**, 169–185.

Jakobsen, K.S., Hughes, D.W. and Galau, G.A. (1994) Simultaneous induction of postabscission and germination mRNAs in cultured dicotyledonous embryos. *Planta* **192**, 384–394.

Jiang, L., Abrams, S.R. and Kermode, A.R. (1996) Vicilin and napin storage-protein gene promoters are responsive to abscisic acid in developing tobacco seed but lose sensitivity following premature desiccation. *Plant Physiology* **110**, 1135–1144.

Judd, W.S., Campbell, C.S., Kellog, E.A. and Stevens, P.F. (1999) *Plant systematics: a phylogenetic approach*. Sunderland, Massachusetts, Sinauer Associates, Inc.

Kamiya, Y. and Garcia-Martinez, J.L. (1999) Regulation or gibberellin biosynthesis by light. *Current Opinion in Plant Biology* **2**, 398–403.

Karssen, C.M. and Laçka, E. (1986) A revision of the hormone balance theory of seed dormancy: Studies on gibberellin and/or abscisic acid-deficient mutants of *Arabidopsis thaliana*. pp. 315–323 *in* Bopp, M. (Ed.) *Plant growth substances 1985.* Berlin, Springer-Verlag.

Kasperbauer, M.J. (1968) Dark-germination of reciprocal hybrid seed from light-requiring and -indifferent *Nicotiana tabacum*. *Physiologia Plantarum* **21**, 1308–1311.

Kelly, K.M., van Staden, J. and Bell, W.E. (1992) Seed coat structure and dormancy. *Plant Growth Regulation* **11**, 201–209.

Kim, J.B., Olek, A.T. and Carpita, N.C. (2000) Cell wall and membrane-associated exo-β-D-glucanases from developing maize seedlings. *Plant Physiology* **123**, 471–485.

Kincaid, R.R. (1935) The effects of certain environmental factors on the germination of Florida cigar-wrapper tobacco seeds. *Technical Bulletin of the University of Florida Agricultural Experimental Station* **277**, 5–47.

Klarzynski, O., Plesse, B., Joubert, J.M., Yvin, J.C., Kopp, M., Kloareg, B. and Fritig, B. (2000) Linear β-1,3-glucans are elicitors of defense responses in tobacco. *Plant Physiology* **124**, 1027–1037.

Koornneef, M., Bentsink, L. and Hilhorst, H. (2002) Seed dormancy and germination. *Current Opinion in Plant Biology* **5**, 33–36.

Krabel, D., Eschrich, W., Wirth, S. and Wolf, G. (1993) Callase-(1,3-β-D-glucanase) activity during spring reactivation in deciduous trees. *Plant Science* **93**, 19–23.

Kretsch, T., Emmler, K. and Schäfer, E. (1995) Spatial and temporal pattern of light-regulated gene expression during tobacco seedling development: The photosystem II-related genes *Lhcb (Cab)* and *PsbP (Oee2)*. *The Plant Journal* **7**, 715–729.

Kunze, I., Kunze, G., Bröker, M., Manteuffel, R., Meins, F. and Müntz, K. (1998) Evidence for secretion of vacuolar α-mannosidase, class I chitinase, and class I β-1,3-glucanase in suspension cultures of tobacco cells. *Planta* **205**, 92–99.

Leah, R., Tommerup, H., Svendsen, I. and Mundy, J. (1991) Biochemical and molecular characterization of three barley seed proteins with antifungal properties. *Journal of Biological Chemistry* **266**, 1564–1573.

Lehmann, K., Hause, B., Altmann, D. and Kock, M. (2001) Tomato ribonuclease LX with the functional endoplasmic reticulum retention motif HDEF is expressed during programmed cell death processes, including xylem differentiation, germination, and senescence. *Plant Physiology* **127**, 436–449.

Leubner-Metzger, G. (2001) Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. *Planta* **213**, 758–763.

Leubner-Metzger, G. (2002) Seed after-ripening and over-expression of class I β-1,3-glucanase confer maternal effects on tobacco testa rupture and dormancy release. *Planta* **215**, 959–968.

Leubner-Metzger, G. and Meins, F. (1999) Functions and regulation of plant β-1,3-glucanases (PR-2). pp. 49–76 *in* Datta, S.K.; Muthukrishnan, S. (Eds) *Pathogenesis-related proteins in plants.* Boca Raton, Florida, CRC Press.

Leubner-Metzger, G. and Meins, F. (2000) Sense transformation reveals a novel role for class I β-1,3-glucanase in tobacco seed germination. *The Plant Journal* **23**, 215–221.

Leubner-Metzger, G. and Meins, F. (2001) Antisense-transformation reveals novel roles for class I β-1,3-glucanase in tobacco seed after-ripening and photodormancy. *Journal of Experimental Botany* **52**, 1753–1759.

Leubner-Metzger, G., Fründt, C., Vögeli-Lange, R. and Meins, F. (1995) Class I β-1,3-glucanase in the endosperm of tobacco during germination. *Plant Physiology* **109**, 751–759.

Leubner-Metzger, G., Fründt, C. and Meins, F. (1996) Effects

of gibberellins, darkness and osmotica on endosperm rupture and class I β-1,3-glucanase induction in tobacco seed germination. *Planta* **199**, 282–288.

Leubner-Metzger, G., Petruzzelli, L., Waldvogel, R., Vögeli-Lange, R. and Meins, F. (1998) Ethylene-responsive element binding protein (EREBP) expression and the transcriptional regulation of class I β-1,3-glucanase during tobacco seed germination. *Plant Molecular Biology* **38**, 785–795.

Li, B.L. and Foley, M.E. (1997) Genetic and molecular control of seed dormancy. *Trends in Plant Science* **2**, 384–389.

Liotenberg, S., North, H. and Marion-Poll, A. (1999) Molecular biology and regulation of abscisic acid biosynthesis in plants. *Plant Physiology and Biochemistry* **37**, 341–350.

Liptay, A. and Schopfer, P. (1983) Effect of water stress, seed coat restraint, and abscisic acid upon different germination capabilities of two tomato lines at low temperature. *Plant Physiology* **73**, 935–938.

Livne, B., Faktor, O., Zeitoune, S., Edelbaum, O. and Sela, I. (1997) TMV-induced expression of tobacco β-glucanase promoter activity is mediated by a single, inverted, GCC motif. *Plant Science* **130**, 159–169.

Marin, E., Nussaume, L., Quesada, A., Gonneau, M., Sotta, B., Hugueney, P., Frey, A. and Marion-Poll, A. (1996) Molecular identification of zeaxanthin epoxidase of *Nicotiana plumbaginifolia*, a gene involved in abscisic acid biosynthesis and corresponding to the ABA locus of *Arabidopsis thaliana*. *EMBO Journal* **15**, 2331–2342.

Matzke, A.J.M., Stoger, E.M. and Matzke, M.A. (1993) A zein gene promoter fragment drives GUS expression in a cell layer that is interposed between the endosperm and the seed coat. *Plant Molecular Biology* **22**, 553–554.

Meikle, P.J., Bonig, I., Hoogenraad, N.J., Clarke, A.E. and Stone, B.A. (1991) The location of (1,3)-β-glucans in the walls of pollen tubes of *Nicotiana alata* using a (1,3)-β-glucan-specific monoclonal antibody. *Planta* **185**, 1–8.

Meikle, P.J., Hoogenraad, N.J., Bonig, I., Clarke, A.E. and Stone, B.A. (1994) A (1→3, 1→4)-β-glucan-specific monoclonal antibody and its use in the quantification and immunocytochemical location of (1→3, 1→4)-β-glucans. *The Plant Journal* **5**, 1–9.

Meins, F., Neuhaus, J.-M., Sperisen, C. and Ryals, J. (1992) The primary structure of plant pathogenesis-related glucanohydrolases and their genes. pp. 245–282 *in* Boller, T.; Meins, F. (Eds) *Genes involved in plant defense.* Vienna, Springer-Verlag.

Mella, R.A., Maldonado, S. and Sanchez, R.A. (1994) Phytochrome-induced structural changes and protein degradation prior to radicle protrusion in *Datura ferox* seeds. *Canadian Journal of Botany* **73**, 1371–1378.

Mo, B.X. and Bewley, J.D. (2002) β-Mannosidase (EC 3.2.1.25) activity during and following germination of tomato (*Lycopersicon esculentum* Mill.) seeds. Purification, cloning and characterization. *Planta* **215**, 141–152.

Mohapatra, S.C. and Johnson, W.H. (1978) Development of the tobacco seedling. 1. Relationship between moisture uptake and light sensitivity during seed germination in a flue-cured variety. *Tobacco Research* **4**, 41–49.

Morohashi, Y. (2002) Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion. *Journal of Experimental Botany* **53**, 1643–1650.

Morohashi, Y. and Matsushima, H. (2000) Development of β-1,3-glucanase activity in germinated tomato seeds. *Journal of Experimental Botany* **51**, 1381–1387.

Nguyen, H., Brown, R.C. and Lemmon, B.E. (2002) Cytoskeletal organization of the micropylar endosperm in *Coronopus didymus* L. (*Brassicaceae*). *Protoplasma* **219**, 210–220.

Nonogaki, H., Gee, O.H. and Bradford, K.J. (2000) A germination-specific endo-β-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. *Plant Physiology* **123**, 1235–1245.

Ohta, M., Ohme-Takagi, M. and Shinshi, H. (2000) Three ethylene-responsive transcription factors in tobacco with distinct transactivation functions. *The Plant Journal* **22**, 29–38.

Ori, N., Sessa, G., Lotan, T., Himmelhoch, S. and Fluhr, R. (1990) A major stylar matrix polypeptide (Sp41) is a member of the pathogenesis-related proteins superclass. *EMBO Journal* **9**, 3429–3436.

Osmond, R.I.W., Hrmova, M., Fontaine, F., Imberty, A. and Fincher, G.B. (2001) Binding interactions between barley thaumatin-like proteins and (1,3)-β-D-glucans – Kinetics, specificity, structural analysis and biological implications. *European Journal of Biochemistry* **268**, 4190–4199.

Peng, J. and Harberd, N.P. (2002) The role of GA-mediated signalling in the control of seed germination. *Current Opinion in Plant Biology* **5**, 376–381.

Petruzzelli, L., Kunz, C., Waldvogel, R., Meins, F. and Leubner-Metzger, G. (1999) Distinct ethylene- and tissue-specific regulation of β-1,3-glucanases and chitinases during pea seed germination. *Planta* **209**, 195–201.

Petruzzelli, L., Coraggio, I. and Leubner-Metzger, G. (2000) Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclopropane-1-carboxylic acid oxidase. *Planta* **211**, 144–149.

Phillips, J., Artsaenko, O., Fiedler, U., Horstmann, C., Mock, H.P., Müntz, K. and Conrad, U. (1997) Seed-specific immunomodulation of abscisic acid activity induces a developmental switch. *EMBO Journal* **16**, 4489–4496.

Ren, C.W. and Kermode, A.R. (2000) An increase in pectin methyl esterase activity accompanies dormancy breakage and germination of yellow cedar seeds. *Plant Physiology* **124**, 231–242.

Rezzonico, E., Flury, N., Meins, F. and Beffa, R. (1998) Transcriptional down-regulation by abscisic acid of pathogenesis-related β-1,3-glucanase genes in tobacco cell cultures. *Plant Physiology* **117**, 585–592.

Rinne, P.L.H., Kaikuranta, P.M. and van der Schoot, C. (2001) The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *The Plant Journal* **26**, 249–264.

Scherp, P., Grotha, R. and Kutschera, U. (2001) Occurrence and phylogenetic significance of cytokinesis-related callose in green algae, bryophytes, ferns and seed plants. *Plant Cell Reports* **20**, 143–149.

Schmid, M., Simpson, D. and Gietl, C. (1999) Programmed cell death in castor bean endosperm is associated with the accumulation and release of a cysteine endopeptidase from ricinosomes. *Proceedings of the National Academy of Sciences, USA* **96**, 14159–14164.

Schopfer, P. (2001) Hydroxyl radical-induced cell-wall loosening *in vitro* and *in vivo*: implications for the control of elongation growth. *The Plant Journal* **28**, 679–688.

Schopfer, P. and Plachy, C. (1984) Control of seed germination by abscisic acid. II. Effect on embryo water uptake in *Brassica napus* L. *Plant Physiology* **76**, 155–160.

Schopfer, P. and Plachy, C. (1993) Photoinhibition of radish (*Raphanus sativus* L.) seed germination – Control of growth potential by cell-wall yielding in the embryo. *Plant, Cell and Environment* **16**, 223–229.

Schopfer, P., Plachy, C. and Frahry, G. (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiology* **125**, 1591–1602.

Seetharaman, K., Whitehead, E., Keller, N.P., Waniska, R.D. and Rooney, L.W. (1997) *In vitro* activity of *Sorghum* seed antifungal proteins against grain mold pathogens. *Journal of Agricultural and Food Chemistry* **45**, 3666–3671.

Simmons, C.R. (1994) The physiology and molecular biology of plant 1,3-β-D-glucanases and 1,3;1,4-β-D-glucanases. *Critical Reviews in Plant Sciences* **13**, 325–387.

Sivaguru, M., Fujiwara, T., Samaj, J., Baluska, F., Yang, Z., Osawa, H., Maeda, T., Mori, T., Volkmann, D. and Matsumoto, H. (2000) Aluminium-induced 1,3-β-D-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of alluminium toxicity in plants. *Plant Physiology* **124**, 991–1005.

Sova, V.V., Zvyagintseva, T.N., Svetasheva, T.G., Burtseva, Y.V. and Elyakova, L.A. (1997) Comparative characterization of hydrolysis and transglycosylation catalyzed by β-1,3-glucanases from various sources. *Biochemistry (Moscow)* **62**, 1113–1118.

Sreenivasulu, N., Altschmied, L., Panitz, R., Hahnel, U., Michalek, W., Weschke, W. and Wobus, U. (2002) Identification of genes specifically expressed in maternal and filial tissues of barley caryopses: A cDNA array analysis. *Molecular Genetics and Genomics* **266**, 758–767.

Still, D.W. and Bradford, K.J. (1997) Endo-β-mannanase activity from individual tomato endosperm caps and radicle tips in relation to germination rates. *Plant Physiology* **113**, 21–29.

Suzuki, M., Miyamoto, R., Hattori, T., Nakamura, K. and Asahi, T. (1995) Differential regulation of the expression in transgenic tobacco of the gene for β-glucuronidase under the control of the 5′-upstream regions of two catalase genes from castor bean. *Plant and Cell Physiology* **36**, 273–279.

Toorop, P.E., Bewley, J.D. and Hilhorst, H.W.M. (1996) Endo-β-mannanase isoforms are present in the endosperm and embryo of tomato seeds, but are not essentially linked to the completion of germination. *Planta* **200**, 153–158.

Toorop, P.E., van Aelst, A.C. and Hilhorst, H.W.M. (1998) Endosperm cap weakening and endo-β-mannanase activity during priming of tomato (*Lycopersicon esculentum* cv. Moneymaker) seeds are initiated upon crossing a threshold water potential. *Seed Science Research* **8**, 483–491.

Toorop, P.E., van Aelst, A.C. and Hilhorst, H.W.M. (2000) The second step of the biphasic endosperm cap weakening that mediates tomato (*Lycopersicon esculentum*) seed germination is under control of ABA. *Journal of Experimental Botany* **51**, 1371–1379.

Toyomasu, T., Kawaide, H., Mitsuhashi, W., Inoue, Y. and Kamiya, Y. (1998) Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. *Plant Physiology* **118**, 1517–1523.

Visser, K., Vissers, A.P.A., Cagirgan, M.I., Kijne, J.W. and Wang, M. (1996) Rapid germination of a barley mutant is correlated with a rapid turnover of abscisic acid outside the embryo. *Plant Physiology* **111**, 1127–1133.

Vögeli-Lange, R., Fründt, C., Hart, C.M., Beffa, R., Nagy, F. and Meins, F. (1994) Evidence for a role of β-1,3-glucanase in dicot seed germination. *The Plant Journal* **5**, 273–278.

Watkins, J.T., Cantliffe, D.J., Huber, D.J. and Nell, T.A. (1985) Gibberellic acid stimulated degradation of endosperm in pepper. *Journal of the American Society for Horticultural Science* **110**, 61–65.

Welbaum, G.E., Bradford, K.J., Yim, K.-O., Booth, D.T. and Oluoch, M.O. (1998) Biophysical, physiological and biochemical processes regulating seed germination. *Seed Science Research* **8**, 161–172.

Wittich, P.E. and Graven, P. (1998) Callose deposition and breakdown, followed by phytomelan synthesis in the seed coat of *Gasteria verrucosa* (Mill.) H. Duval. *Protoplasma* **201**, 221–230.

Worrall, D., Hird, D.L., Hodge, R., Paul, W., Draper, J. and Scott, R. (1992) Premature dissolution of the microsporocyte callose wall causes male sterility in transgenic tobacco. *The Plant Cell* **4**, 759–771.

Wu, C.-T. and Bradford, K.J. (2002) Class I chitinase is expressed specifically in the micropylar region of *gib-1* tomato seeds in response to wounding or methyl jasmonate. Abstract, Seventh International Workshop on Seeds, Salamanca, Spain.

Wu, C.-T., Leubner-Metzger, G., Meins, F. and Bradford, K.J. (2000) Class I β-1,3-glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radicle emergence. *Plant Physiology* **126**, 1299–1313.

Yamaguchi, S., Kamiya, Y. and Sun, T.P. (2001) Distinct cell-specific expression patterns of early and late gibberellin biosynthetic genes during *Arabidopsis* seed germination. *The Plant Journal* **28**, 443–453.

Yamaguchi-Shinozaki, K., Mino, M., Mundy, J. and Chua, N.-H. (1990) Analysis of an ABA-responsive rice gene promoter in transgenic tobacco. *Plant Molecular Biology* **15**, 905–912.

Yim, K.O. and Bradford, K.J. (1998) Callose deposition is responsible for apoplastic semipermeability of the endosperm envelope of muskmelon seeds. *Plant Physiology* **118**, 83–90.

Young, T.E. and Gallie, D.R. (2000) Regulation of programmed cell death in maize endosperm by abscisic acid. *Plant Molecular Biology* **42**, 397–414.

Received 20 February 2002
accepted after revision 8 November 2002