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INVITED REVIEW

Plant hormone interactions during seed dormancy release and germination

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Abstract

This review focuses mainly on eudicot seeds, and on the interactions between abscisic acid (ABA), gibberellins (GA), ethylene, brassinosteroids (BR), auxin and cytokinins in regulating the interconnected molecular processes that control dormancy release and germination. Signal transduction pathways, mediated by environmental and hormonal signals, regulate gene expression in seeds. Seed dormancy release and germination of species with coat dormancy is determined by the balance of forces between the growth potential of the embryo and the constraint exerted by the covering layers, e.g. testa and endosperm. Recent progress in the field of seed biology has been greatly aided by molecular approaches utilizing mutant and transgenic seeds of *Arabidopsis thaliana* and the *Solanaceae* model systems, tomato and tobacco, which are altered in hormone biology. ABA is a positive regulator of dormancy induction and most likely also maintenance, while it is a negative regulator of germination. GA releases dormancy, promotes germination and counteracts ABA effects. Ethylene and BR promote seed germination and also counteract ABA effects. We present an integrated view of the molecular genetics, physiology and biochemistry used to unravel how hormones control seed dormancy release and germination.

Keywords: abscisic acid, after-ripening, *Arabidopsis*, auxin, brassinosteroid, coat dormancy, cytokinin, endosperm-limited germination, ethylene, gibberellin, hormone mutants, *Nicotiana*, seed dormancy, seed germination, signal transduction pathways, transcription factors

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Introduction

The German botanist Julius von Sachs (1832–1897) proposed that plants produce, transport and perceive 'organ-forming substances' responsible for the formation and growth of different plant organs. The plant hormones abscisic acid (ABA), gibberellins (GA), ethylene, brassinosteroids (BR), auxins, cytokinins and other signalling molecules have profound effects on plant development at vanishingly low concentrations. They are chemical messengers for the communication among cells, tissues and organs of higher plants. The seeds of higher plants contain an embryo surrounded by covering layers and function to ensure the establishment of a new plant generation. Plant hormones are extremely important for the regulation of seed dormancy and germination (Fig. 1, Koornneef *et al.*, 2002; Finkelstein, 2004). Little is known about the interconnected key molecular processes controlling dormancy and germination in response to hormonal and environmental cues.

Seed dormancy is a temporary failure or block of a viable seed to complete germination under physical conditions that normally favour this (reviewed by Hilhorst, 1995; Bewley, 1997b; Li and Foley, 1997; Baskin and Baskin, 2004). Two major forms of physiological seed dormancy have been described, namely embryo and coat dormancy (sometimes termed coat-enhanced dormancy). Germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expansion. This usually

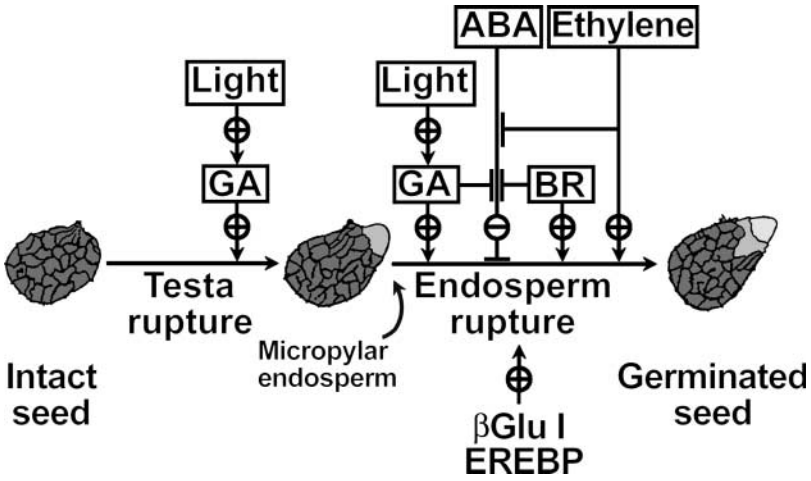

Figure 1. Hormonal interactions during tobacco seed dormancy release and germination and their effects on testa and endosperm rupture. Dormancy release involves the light/gibberellin (GA) pathway and results in testa rupture. The light/GA pathway also promotes endosperm rupture. Class I β-1,3-glucanase (βGlu I) and other hydrolases are induced by the light/gibberellin (GA) pathway in the micropylar endosperm and facilitate endosperm rupture and radicle protrusion. Endosperm-specific βGlu I expression and endosperm rupture are inhibited by abscisic acid (ABA). The light/GA pathway also counteracts ABA effects by promoting ABA degradation. Ethylene and brassinosteroids (BR) counteract ABA effects and promote endosperm rupture, but do not affect testa rupture. EREBPs (ethylene responsive element binding proteins) are transcription factors that mediate hormonal regulation of βGlu I expression and endosperm rupture. BR and light/GA promote tobacco endosperm rupture by distinct signal transduction pathways. In addition, seed after-ripening is associated with a decrease in ABA content and sensitivity, and an increase in GA sensitivity or loss of GA requirement. A 'plus' sign means promotion and a 'minus' sign inhibition.

culminates in rupture of the covering layers and emergence of the radicle, generally considered as the completion of the germination process. Radicle protrusion at the completion of seed germination depends on embryo growth driven by water uptake. Uptake of water by a seed is triphasic, with a rapid initial uptake (phase I, i.e. imbibition) followed by a plateau phase (phase II). A further increase in water uptake (phase III) occurs only when germination is completed, as the embryo axis elongates and breaks through its covering structures (Schopfer and Plachy, 1984; Bewley, 1997b; Manz *et al.*, 2005). Cell elongation is necessary, and is generally accepted to be sufficient, for the completion of radicle protrusion; cell division is not essential (Barroco *et al.*, 2005).

In many plant species the seed-covering layers impose a physical constraint to radicle protrusion, which has to be overcome by the growth potential of the embryo. In non-endospermic seeds and in *Arabidopsis*, with only one cell layer of endosperm, the testa (seed coat, diploid maternal tissue) characteristics are mainly responsible for this coat dormancy (Debeaujon and Koornneef, 2000; Debeaujon *et al.*, 2000). In many mature non-endospermic seeds, the cotyledons are the sole storage organs, complete endosperm assimilation occurs during seed development, and the embryo is enclosed by the testa as the only covering layer. The testa imposes a restraint to radicle protrusion in radish (Schopfer and Plachy, 1993) and *Arabidopsis* (Debeaujon and Koornneef, 2000; Debeaujon *et al.*, 2000; Rajjou *et al.*, 2004), but is no hindrance to radicle protrusion in rape (Schopfer and Plachy, 1984) and pea (Petruzzelli *et al.*, 2000). In addition to the testa, the covering layers of endospermic seeds include the endosperm, which is usually triploid in angiosperms, with two-thirds of its genome of maternal origin. In endospermic seeds the contributions of both the testa and the endosperm layers to the extent of coat dormancy have to be considered (reviewed by Hilhorst, 1995; Bewley, 1997a; Leubner-Metzger, 2003b). Endosperm rupture is the main germination-limiting process in seeds of the *Asteraceae* (e.g. lettuce), *Solanaceae* (e.g. tomato and tobacco) and *Rubiaceae* (e.g. coffee). In these cases of endosperm-limited germination, weakening of the micropylar endosperm surrounding the radicle tip appears to be required for radicle protrusion, and is likely to involve cell-wall hydrolysis by hydrolytic enzymes. Major recent contributions to our understanding of endosperm-limited germination have come from research on tomato and *Nicotiana* species (reviewed by Hilhorst, 1995; Bewley, 1997a; Koornneef *et al.*, 2002; Leubner-Metzger, 2003b).

However, recent progress in wider aspects of seed biology has been enhanced greatly by molecular genetic studies with *Arabidopsis thaliana* hormone mutants.

This review focuses mainly on eudicot seeds, and on the interactions between ABA, GA, ethylene and BR in controlling the interconnected molecular processes of dormancy release and germination (Figs 1 and 2). We present an overview on how molecular genetics, physiology and biochemistry are helping to unravel the basis of germination and dormancy in seed biology.

Abscisic acid (ABA): a positive regulator of dormancy induction, a negative regulator of germination

Development of 'orthodox seeds' is completed by maturation and desiccation, when storage compounds have accumulated, water content decreases, ABA accumulates, and desiccation tolerance and primary dormancy are established. No receptor for the perception of ABA has been identified conclusively (Finkelstein *et al.*, 2002). However, possible candidates are known (Razem *et al.*, 2004; Osakabe *et al.*, 2005). RPK1, a leucine-rich repeat receptor-like protein kinase, may be a key membrane-bound regulator of early ABA signalling in seeds and seedlings; *RPK1* knockout mutant seeds exhibit decreased ABA sensitivity of germination (Table 1). In many plant species endogenous ABA is involved in the induction, and perhaps in the maintenance, of the dormant state (reviewed by Hilhorst, 1995; Bewley, 1997b; Koornneef *et al.*, 2002; Leubner-Metzger, 2003b; Nambara and Marion-Poll, 2003). Overexpression of genes for ABA biosynthesis can increase seed ABA content and enhance seed dormancy or delay germination (Frey *et al.*, 1999; Thompson *et al.*, 2000; Lindgren *et al.*, 2003; Nambara and Marion-Poll, 2003). Enhanced dormancy is also evident in *Arabidopsis cyp707a2* mutants with increased ABA content due to a block of seed ABA catabolism (ABA 8' hydroxylase, Table 1, Kushiro *et al.*, 2004). The abscisic aldehyde oxidase 3 (AAO3) protein of *Arabidopsis* catalyses the final step in ABA biosynthesis and is a target of a self-regulatory loop regulating ABA biosynthesis (Xiong

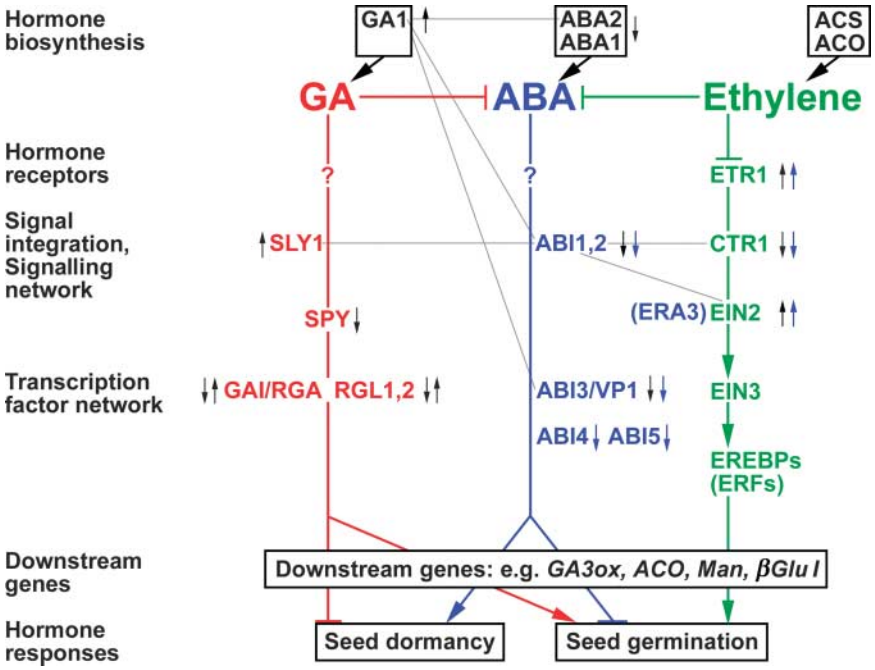

Figure 2. Schematic representation of the interactions between the gibberellin (GA), abscisic acid (ABA) and ethylene signalling pathways in the regulation of seed dormancy and germination. The model is mainly based on *Arabidopsis* hormone mutant analyses, the positions of some components are speculative, and details are explained in the text. Promotion or inhibition is indicated by thick arrows and blocks, respectively. Interactions based on extragenic suppressor or enhancer screens are indicated by thin grey lines. Small black arrows indicate enhancement (up-arrow) or reduction (down-arrow) of seed dormancy, and small blue arrows indicate enhancement or reduction of seed ABA sensitivity upon mutation of the corresponding protein. Corresponding hormone mutants *of Arabidopsis thaliana*: *aba1*, *aba2* = *ABA-deficient1,2*; *abi1* to *abi5* = *ABA-insensitive1* to *ABA-insensitive5*; *ctr1* = *constitutive triple response1*; *ein2*, *ein3* = *ethylene insensitive2*, *3*; *era3* = *enhanced response to ABA3*; *gai* = *GA-insensitive*; *sly1* = *sleepy1*; *spy* = *spindly*; *rga* = *repressor-of-ga1-3*; *rgl 1*, *rgl2* = *rga-like1*, *2*. Other abbreviations: ACO = ACC oxidase; ACS = ACC synthase; EREBP = ethylene responsive element binding protein; ERF = ethylene responsive factor; GA3ox = GA 3-oxidase; Man = mannanase; βGlu I = class I β-1,3-glucanase; *vp1* = *viviparous1* (maize mutant). (From Basra, 2005a with permission)

Table 1. Selected plant hormone mutants and their seed phenotypes. Hormone mutants of *Arabidopsis thaliana*, *Lycopersicon esculentum*, *Nicotiana tabacum*, *Nicotiana plumbaginifolia* and *Zea mays* are grouped in defined classes for abscisic acid (ABA), gibberellin (GA), ethylene (C_2H_4) and brassinosteroids (BR)

Hormone	Mutant class[a]	Species	Gene/locus[b]	Protein function[c]	Mutant[d] or transgenic line	Dominance[e]	Mutant seed dormancy	Select. other mutant seed effects	References [f]
ABA	Deficiency	*A. thaliana*	*ABA1*	ABA biosynthesis	*aba1-1*	R	Reduced	Vivipary	13, 24, 26, 27
		L. esculentum	*NOT*	ABA biosynthesis	*not*	R	Reduced	Vivipary	13, 19, 54
			SIT [W]	ABA biosynthesis	*sit* [W]	R	Reduced	Vivipary	
		Z. mays	*VP14*	Carotinoid cleavage, ABA biosynthesis	*vp14*	R	Reduced	Vivipary	31, 55
		N. tabacum		Anti-ABA antibody			Reduced		42
		N. plumbaginifolia	*ABA2*	ABA biosynthesis	*aba2*	R	Reduced	Vivipary	14, 15, 34
				ABA biosynthesis	antisense-ABA2	D	Reduced		
	Overproduction	*A. thaliana*	*CYP707A*	ABA degradation	*cyp707a2*	R	Enhanced		27
		N. plumbaginifolia	*ABA2*	ABA biosynthesis	sense-ABA2	D	Enhanced		14
	Insensitivity	*A. thaliana*	*ABI1*	Ser/Thr protein phosphatases 2C (PP2C)	*abi1-1*	SD	Reduced	Reduced ABA sensitivity of germination	2, 26, 30, 33, 38, 51
			ABI2	PP2C	*abi2-1*	SD	Reduced		
		A. thaliana	*ABI3*	VP1/ABI3-type B3-domain TF	*abi3*	R	Severely reduced	Reduced ABA sensitivity of germination; reduced seed longevity	1, 21, 30, 33, 36, 38, 43, 45
		A. thaliana	*ABI4*	AP2/EREBP TF	*abi4*	R	Normal	Reduced ABA sensitivity of germination	3, 12, 39, 48
		A. thaliana	*ABI5*	bZIP TF	*abi5*	R	Normal	Reduced ABA sensitivity of germination	3, 11, 12, 32, 33
		A. thaliana	*ABI8*	?	*abi8*	?	?	Reduced ABA sensitivity of germination	5
		A. thaliana	*RPK1*	Leucine-rich repeat receptor-like kinase	*rpk1*	R	Reduced (?)	Reduced ABA sensitivity of germination	39a
		Z. mays	*VP1*	VP1/ABI3-type B3-domain TF	*vp1*	R	Reduced	Vivipary	1, 21, 35, 47

Table 1. *Continued*

Hormone	Mutant class[a]	Species	Gene/locus[b]	Protein function[c]	Mutant[d] or transgenic line	Dominance[e]	Mutant seed dormancy	Select. other mutant seed effects	References [f]
	Hypersensitivity or constitutive response	*A. thaliana*	*ERA1*	Farnesyl transferase	*era1*	R	Enhanced		2, 8, 16, 46
			ERA3	*EIN2* allele	*era3*	R	Enhanced		
		A. thaliana	*SAD1*	Sm-like snRNP protein	*sad1*	R	Enhanced	ABA hypersensitive germination	56
		A. thaliana	*ABH1*	mRNA cap-binding protein	*abh1*	R	?	ABA hypersensitive germination	22
GA	Deficiency	*A. thaliana*	*GA1*	GA biosynthesis	*ga1*	R	Enhanced		9, 25, 26, 41, 44, 51
		L. esculentum	*GIB-1*	GA biosynthesis	*gib-1*	R	Enhanced		20, 25, 26, 44
	Insensitivity	*A. thaliana*	*GAI*	DELLA-type GRAS TF	*gai* gain-of function	SD	Enhanced		10, 26, 41, 44
		A. thaliana	*SLY1*	F-box protein	*sly1*	R	Enhanced		41, 50, 51, 57
	Hypersensitivity or constitutive response	*A. thaliana*	*GAI*	DELLA-type GRAS TF	*gai-t6,* loss-of function	R	Reduced		6, 10, 26, 41, 44
			RGL2	DELLA-type GRAS TF	*rgl2,* loss-of function	R	Reduced		
		A. thaliana	*SPY*	*O*-GlcNAc transferase	*spy*	R	Reduced		18, 23, 41, 52, 54, 57
C_2H_4	Insensitivity	*A. thaliana*	*ETR1*	Ethylene receptor	*etr1-1*	D	Enhanced	ABA hypersensitive germination	2, 4, 16, 17, 29, 40
			EIN2	Metal transporter?	*ein2*	R	Enhanced	ABA hypersensitive germination	2, 17
			EIN3	TF	*ein3*	R	?		49
	Hypersensitivity or constitutive response	*A. thaliana*	*CTR1*	Raf-like protein kinase (MAPKKK)	*ctr1*	R	Slightly reduced	Slightly reduced ABA sensitivity of germination	2, 7, 17, 40

Table 1. *Continued*

Hormone	Mutant class[a]	Species	Gene/locus[b]	Protein function[c]	Mutant[d] or transgenic line	Dominance[e]	Mutant seed dormancy	Select. other mutant seed effects	References [f]
BR	Deficiency	*A. thaliana*	*DET2*	BR biosynthesis	*det2-1*	R		ABA hypersensitive germination	50
	Insensitivity	*A. thaliana*	*BRI1*	BR receptor	*bri1-1*	R		ABA hypersensitive germination	28, 50, 53

[a] Four mutant classes either altered in hormone biosynthesis (hormone-deficient mutants, hormone-overproducing mutants) or in hormone response/signal transduction (hormone-insensitive mutants, constitutive response/hormone-hypersensitive mutants).
[b] Mutant abbreviations: *aba1, aba2 = ABA-deficient1, 2; abh1 = ABA-hypersensitive1; abi1 to abi8 = ABA-insensitive1 to ABA-insensitive8; bri1 = Brassinosteriod-insensitive1; ctr1 = constitutive triple response1; det2-1 = de-etiolated2; ein2, ein3 = ethylene insensitive2, 3; era1, era3 = enhanced response to ABA1, ABA3; etr1 = ethylene resistant1; ga1 = GA-deficient1; gai = GA-insensitive; gib-1 = GA-deficient-1; not = notabilis; sit^{W} = sitiens^{W}; sly1 = sleepy1; spy = spindly; rga = repressor-of-ga1-3; rgl2 = rga-like2; rpk1 = receptor-like protein kinase1; sad1 = supersensitive to ABA and drought; spy = spindly; vp1 = viviparous1*
[c] TF = transcription factor.
[d] In some cases several alleles, in some cases specific alleles, are given.
[e] Dominance over wild-type locus: D = dominant, SD = semi-dominant, R = recessive.
[f] Selected references: 1, Baumbusch *et al.* (2004); 2, Beaudoin *et al.* (2000); 3, Bensmihen *et al.* (2005); 4, Bleecker *et al.* (1988); 5, Brocard-Gifford *et al.* (2004); 6, Cao *et al.* (2005); 7, Chang (2003); 8, Cutler *et al.* (1996); 9, Debeaujon and Koornneef (2000); 10, Derkx and Karssen (1993); 11, Finkelstein and Lynch (2000); 12, Finkelstein *et al.* (2002); 13, Finkelstein *et al.* (1998); 14, Frey *et al.* (1999); 15, Frey *et al.* (2004); 16, Ghassemian *et al.* (2000); 17, Hall *et al.* (2001); 18, Hartweck *et al.* (2002); 19, Hilhorst (1995); 20, Hilhorst and Karssen, 1992; 21, Holdsworth *et al.* (2001); 22, Hugouvieux *et al.* (2002); 23, Izhaki *et al.* (2001); 24, Karssen *et al.* (1983); 25, Karssen *et al.* (1989); 26, Koornneef and Karssen (1994); 27, Kushiro *et al.* (2004); 28, Leubner-Metzger (2001); 29, Leubner-Metzger *et al.* (1998); 30, Leung and Giraudat (1998); 31, Liotenberg *et al.* (1999); 32, Lopez-Molina *et al.* (2001); 33, Lopez-Molina *et al.* (2003); 34, Marin *et al.* (1996); 35, McCarty (1995); 36, Nambara *et al.* (1992); 37, Nambara *et al.* (1998); 38, Nambara *et al.* (2002); 39, Ohta *et al.* (2000); 39a, Osakabe *et al.* (2005); 40, Ouaked *et al.* (2003); 41, Peng and Harberd (2002); 42, Phillips *et al.* (1997); 43, Raz *et al.* (2001); 44, Richards *et al.* (2001); 45, Rohde *et al.* (2000); 46, Ross and O'Neill (2001); 47, Schwechheimer and Bevan (1998); 48, Söderman *et al.* (2000); 49, Solano *et al.* (1998); 50, Steber and McCourt (2001); 51, Steber *et al.* (1998); 52, Swain *et al.* (2001); 53, Szekeres (2003); 54, Thompson *et al.* (2000); 55, White *et al.* (2000); White and Rivin (2000); 56, Xiong *et al.* (2001); 57, Yamaguchi and Kamiya (2002).

et al., 2001). The *aao3* mutants with reduced seed ABA biosynthesis exhibit reduced dormancy (Gonzalez-Guzman *et al.*, 2004).

In contrast to ABA overproduction, ABA deficiency during seed development is associated with the absence of primary dormancy of the mature seed (Table 1). ABA production during seed development can be of dual origin, the embryo and/or the maternal tissues. Reciprocal crosses and/or grafting experiments between wild-type and ABA-deficient mutants of *Arabidopsis* (Karssen *et al.*, 1983; Koornneef and Karssen, 1994; Nambara and Marion-Poll, 2003), tomato (Groot and Karssen, 1992; Hilhorst, 1995) and *Nicotiana plumbaginifolia* (Frey *et al.*, 1999) have shown that only ABA produced by the embryo itself during seed development is necessary to impose a lasting dormancy. Maternal ABA produced by the seed-covering layers, or ABA application (resembling maternal ABA) during seed development, both fail to induce seed dormancy. It is not known if maternal ABA penetrates the embryonic axis, but it is known that it affects aspects of seed development other than dormancy (Finkelstein, 1994; Koornneef and Karssen, 1994). For example, it increases both seed yield and rate of embryo growth in *N. plumbaginifolia* (Frey *et al.*, 2004) and promotes carrot embryo growth (Homrichhausen *et al.*, 2003). This suggests either that the covering layers control embryo growth, or that maternal ABA penetrates the zygotic embryonic axis. Why it does not affect dormancy is unknown.

The formation of non-dormant seeds and precocious germination on the mother plant (vivipary) can occur in ABA-deficient biosynthesis mutants (Table 1), e.g. in *aba1* and *aba2* of *Arabidopsis* (Karssen *et al.*, 1983; Koornneef and Karssen, 1994), *sitiens* (*sit* ^{w}) and *notabilis* (*not*) of tomato (Hilhorst, 1995; Thompson *et al.*, 2000), *aba2* of *N. plumbaginifolia* (Marin *et al.*, 1996), and in several *viviparous* (*vp*) mutants of maize (Liotenberg *et al.*, 1999; White *et al.*, 2000). In the *aba2* mutant of *N. plumbaginifolia*, ABA deficiency is due to a mutation in the *ABA2* gene, encoding zeaxanthin epoxidase, a key step in ABA biosynthesis (Marin *et al.*, 1996). Antisense and sense *ABA2* transformation of *N. plumbaginifolia* results in decreased and increased ABA biosynthesis and seed dormancy, respectively (Table 1, Frey *et al.*, 1999). The onset of dormancy induction in *Nicotiana tabacum* is associated with a peak in ABA at approximately 15–20 d after pollination (DAP), followed by a rapid decline in ABA during later seed maturation. Tobacco dormancy is present in seeds harvested 25 DAP (Yamaguchi-Shinozaki *et al.*, 1990; Jiang *et al.*, 1996; Phillips *et al.*, 1997; Leubner-Metzger, 2005b). Seed dormancy is not established in transgenic tobacco expressing an anti-ABA antibody that causes a deficiency in free ABA (Phillips *et al.*, 1997).

The *Arabidopsis ABA-insensitive* (*abi*) response mutants, *abi1* to *abi5* and *abi8*, have been identified by selecting for seeds capable of germination and good seedling growth in the presence of ABA concentrations that are inhibitory to the wild type (Koornneef and Karssen, 1994; Leung and Giraudat, 1998; Brocard-Gifford *et al.*, 2004). Like the ABA-deficient mutants, several of these ABA-insensitive mutants also exhibit a marked reduction in seed dormancy (Table 1). The original *abi1* mutant seed phenotype was described as having: (1) reduced dormancy, which can be broken by chilling or dry storage; (2) reduced ABA sensitivity of germination; and (3) no precocious germination (Karssen *et al.*, 1990). The *ABI1* and *ABI2* genes encode paralogous serine/threonine protein phosphatases 2C (PP2C) (Leung and Giraudat, 1998; Beaudoin *et al.*, 2000; Schweighofer *et al.*, 2004). The *abi1-1* and *abi2-1* mutations are dominant and lead to the ABA-insensitive seed phenotype and to vegetative responses. In contrast to the gain of function mutant allele *abi1-1* response, loss-of-function alleles of *ABI1* lead to an ABA-hypersensitive response, indicating that the ABI1 PP2C is a negative regulator of ABA responses. However, results from overexpression and microinjection experiments are not compatible with a negative regulatory role of ABI1 (reviewed in Schweighofer *et al.*, 2004). Transcripts of a PP2C are synergistically up-regulated by ABA and calcium in dormant seeds of *Fagus sylvatica* (Lorenzo *et al.*, 2002).

The seed responses of strong alleles of the *Arabidopsis ABI3* gene (Table 1) are severe compared to the *abi1*, *abi2* and the ABA-deficient mutant alleles (Leung and Giraudat, 1998; Schwechheimer and Bevan, 1998; Raz *et al.*, 2001; Finkelstein, 2004). *ABI3* gene promoter activity has been detected in seeds and in vegetative meristems (Rohde *et al.*, 1999; Ng *et al.*, 2004). *ABI3* might play a major role in seed and bud dormancy (Rohde *et al.*, 2000). The ABA-insensitive *viviparous1* (*vp1*) mutant of maize is characterized by severe seed responses, including reduced sensitivity of germination to exogenous ABA and vivipary (e.g. McCarty, 1995; Li and Foley, 1997; Schwechheimer and Bevan, 1998). The *Arabidopsis ABI3* and the maize *VP1* are orthologous genes that encode transcription factors of the B3 domain class that are essential for ABA action. Other members of this class influencing germination include the wild oat *AfVP1* (Jones *et al.*, 2000), and the *Arabidopsis FUS3* and *LEC2* (Rohde *et al.*, 2000; Mönke *et al.*, 2004). Binding of ABI3 and FUS3 to the RY motif located in promoter elements has been shown (Mönke *et al.*, 2004); the B3 motif is both necessary and sufficient for DNA binding of both transcription factors. VP1/ABI3-like proteins can function as activators and repressors of ABA-dependent and -independent gene expression in seeds (e.g. Ezcurra *et al.*, 2000; Holdsworth *et al.*, 2001; Suzuki *et al.*, 2001; Clerkx *et al.*, 2003; Zeng *et al.*, 2003). Ectopic expression of *ABI3* or *VP1* in *Arabidopsis*

confers ABA- and seed-specific responses to vegetative tissues (Leung and Giraudat, 1998; Suzuki *et al.*, 2001; Ng *et al.*, 2004), suggesting that the VP1/ABI3-like proteins are multifunctional transcription factors that integrate ABA and other regulatory signals of seed maturation and developmental arrest. However, expression studies in severely and moderately dormant ecotypes of *Arabidopsis* suggest that ABI3 is not the major immediate regulator required for the establishment of dormancy (Baumbusch *et al.*, 2004). Factors that interact with VP1/ABI3 have been identified, and it has been proposed that the VP1/ABI3-like transcription factors not only regulate seed responses, but function as general regulators for the timing of developmental transitions throughout the life cycle of plants (Hobo *et al.*, 1999; Rohde *et al.*, 2000; Holdsworth *et al.*, 2001). Post-translational targeting of ABI3 for protein degradation (Zhang *et al.*, 2005) and perhaps also farnesylation of ABI3 (Brady *et al.*, 2003) are mechanisms to regulate ABI3-mediated ABA signalling. ABA induction of *ABI1* and *ABI2*, possible negative regulators of ABA signaling, is inhibited by VP1/ABI3 in a feed-forward pathway (Suzuki *et al.*, 2003). Mutation alleles like *abi3-4* show reduced seed longevity and reduced chlorophyll breakdown, which may be partially related to the reduced seed dormancy phenotype (Clerkx *et al.*, 2003).

In another feed-forward pathway, VP1/ABI3 activates ABI5 (Table 1), a positive regulator of ABA signalling (Nambara and Marion-Poll, 2003; Suzuki *et al.*, 2003; Nakabayashi *et al.*, 2005). The *Arabidopsis ABI5* gene encodes a basic leucine zipper (bZIP) transcription factor that binds to the ABA responsive *cis*-acting element (ABRE) of ABA-induced gene promoters. Physical interaction of ABI3 and ABI5 has been demonstrated and appears to be important for the transcriptional activation of genes. Recently, a nuclear protein, Imbibition-inducible 1 (IBM1) was identified that promotes germination of *Arabidopsis* seeds by negatively regulating the ABA transduction pathway (Duque and Chua, 2003). Loss-of-function *imb1* mutants have higher ABI5 contents than wild type. ABI5 is important in determining ABA responsiveness during embryogenesis, seed maturation and regulating the transition after germination to vegetative growth (Finkelstein and Lynch, 2000; Lopez-Molina *et al.*, 2001, 2002; Bensmihen *et al.*, 2002, 2005; Lu *et al.*, 2002). A post-germination developmental arrest checkpoint is mediated by ABA and requires the ABI5 transcription factor. Degradation of ABI5 is regulated by interaction with the ABI five binding protein (AFP), which itself is induced by ABA. In *Arabidopsis*, two mutant alleles, *afp-1* and *afp-2*, have been identified that confer hypersensitivity to ABA (Lopez-Molina *et al.*, 2003).

The transition from water uptake (phases 1 and 2) during germination to water uptake during post-germination growth (phase 3) is inhibited by ABA. ABA inhibits phase 3 water uptake, endosperm rupture, further embryo extension and seedling growth after radicle emergence. However, ABA does not inhibit initial imbibition of water, initial embryo extension growth or testa rupture, e.g. of rape (Schopfer and Plachy, 1984), *N. plumbaginifolia* (Frey *et al.*, 2004), *N. tabacum* (Manz *et al.*, 2005), carrot (Homrichhausen *et al.*, 2003), and *Arabidopsis* (Müller *et al.*, 2005). ABA may inhibit embryo growth by regulating ion-channel activities, aquaporin abundance or other tissue-specific alterations of water uptake (Gao *et al.*, 1999; Manz *et al.*, 2005). The inhibition by ABA of *Arabidopsis* and tobacco seed germination is not a consequence of inhibition of storage lipid mobilization (Pritchard *et al.*, 2002; Penfield *et al.*, 2004; Manz *et al.*, 2005). Two independent programmes appear to operate; one is inhibited by ABA and governs developmental growth resulting in germination, and a second, largely ABA-independent, programme governs storage lipid mobilization.

Arabidopsis ABI4 (Table 1) shows the greatest sequence homology with ethylene-responsive element binding protein (EREBP)-type transcription factors, and contains an APETALA2 (AP2)-like DNA binding domain characteristic of AP2/EREBP family transcriptional regulators (Finkelstein *et al.*, 1998; Ohta *et al.*, 2000; Söderman *et al.*, 2000). ABI4 is likely to be seed-specific and may act downstream of ABI3 or in a parallel pathway. ABA signal transduction via ABI4 is also involved in responses of seeds to sugars (Wobus and Weber, 1999; Huijser *et al.*, 2000; Finkelstein and Gibson, 2002; Finkelstein, 2004). High sugar induces both ABA biosynthesis and expression of *ABI3*, *ABI4* and *ABI5*. Germination of the *abi4* mutant is insensitive to ABA and sugar; their signalling occurs in either parallel or intersecting pathways. ABI3, ABI4 and ABI5 interact, and it is proposed that these transcription factors are key factors in a signalling network that regulates ABA-related seed responses (Brocard-Gifford *et al.*, 2003; Finkelstein, 2004; Katagiri *et al.*, 2005). Another component of this signaling pathway is *MARD1* (*Mediator of ABA-regulated dormancy 1*). *Mard1* mutant seeds are insensitive to external ABA, are less dormant than wild-type seeds and are able to germinate in complete darkness (He and Gan, 2004).

Several *Arabidopsis* ABA-hypersensitive response mutants (Table 1), including *enhanced response to ABA1* (*era1*) and *supersensitive to ABA and drought (sad1)*, exhibit enhanced seed dormancy (Cutler *et al.*, 1996; Ghassemian *et al.*, 2000; Xiong *et al.*, 2001; Brady *et al.*, 2003). Their germination is inhibited by low ABA concentrations that do not affect the wild type. The *ERA1* gene encodes the β-subunit of a farnesyl transferase, and it is proposed that a negative

regulator of ABA sensitivity is modulated by protein farnesylation. The *SAD1* gene encodes a Sm-like U6 small nuclear riboprotein and appears to be involved in the self-regulatory loop that regulates ABA biosynthesis by affecting the *AAO3* transcript content. Germination of the *ABA-hypersensitive1 (abh1)* mutant is also hypersensitive to ABA, and *ABH1* encodes an mRNA cap-binding protein (Hugouvieux *et al.*, 2002). Further proteins that are associated with RNA-dependent processes in germinating seeds are two ribosomal proteins (Toorop *et al.*, 2005). These findings are in agreement with the view that translation of stored mRNAs is required for seed germination and that *de novo* mRNA synthesis controls germination rate and onset (Rajjou *et al.*, 2004). Two other ABA hypersensitive response mutants, *atpirin1* and *gpa1* are impaired in a G-protein-coupled signal transduction pathway (Lapik and Kaufman, 2003), and the *ABA-hypersensitive germination (ahg)* mutants exhibit an altered ABI5 expression pattern (Nishimura *et al.*, 2004). However, not all *Arabidopsis* mutants showing reduced dormancy are altered in ABA biosynthesis or sensitivity (e.g. Leon-Kloosterziel *et al.*, 1996; Papi *et al.*, 2000; Peeters *et al.*, 2002).

ABA is not only a positive regulator of dormancy induction; it also inhibits seed germination, has a role during after-ripening and has been proposed to be a positive regulator of dormancy maintenance. After-ripening, i.e. a period of dry storage at room temperature of freshly harvested, mature seeds, is a common method to facilitate dormancy release (Bewley, 1997b; Leubner-Metzger, 2003b). A decline in ABA content, decreased sensitivity to ABA and increased sensitivity to gibberellins (GA) are associated with the after-ripening-mediated transition from the dormant to the non-dormant state of many species (e.g. Hilhorst, 1995; Benech-Arnold *et al.*, 1999; Beaudoin *et al.*, 2000; Grappin *et al.*, 2000; Romagosa *et al.*, 2001; Koornneef *et al.*, 2002; Leubner-Metzger, 2002; Schmitz *et al.*, 2002; Ali-Rachedi *et al.*, 2004; Feurtado *et al.*, 2004). An exception is the seed of *Avena fatua*, in which ABA and after-ripening, but not GA, are the primary regulators of seed dormancy (Fennimore and Foley, 1998). High ABA contents are present in the strongly dormant seeds of an *Arabidopsis* ecotype (Cape Verde Island, Cvi), but not in after-ripened, non-dormant ones (Ali-Rachedi *et al.*, 2004). The carotenoid biosynthesis inhibitors, norflurazon and fluridone, have been used in experiments to inhibit ABA biosynthesis (e.g. Grappin *et al.*, 2000; Ali-Rachedi *et al.*, 2004; Chae *et al.*, 2004; da Silva *et al.*, 2004). Grappin *et al.* (2000) demonstrated that freshly harvested *N. plumbaginifolia* seeds have higher ABA content and sensitivity. Moreover, *de novo* ABA biosynthesis occurs in imbibed fresh, but not imbibed after-ripened seeds. This *de novo* ABA biosynthesis occurs very early upon imbibition of fresh seeds. Grappin *et al.* (2000) also added gibberellins (GA) or fluridone to the seeds, both of which prevented ABA biosynthesis and accelerated the rate of germination. Simultaneous addition of GA + ABA or fluridone + ABA negated the germination-promoting effects. Expression studies of ABA-regulated genes in *N. tabacum* seeds are in agreement with decreased ABA content and sensitivity in after-ripened seeds, and suggest a common role for ABA during the after-ripening-mediated release from dormancy and control of germination rate of *Nicotiana* species (Leubner-Metzger and Meins, 2000; Leubner-Metzger, 2002, 2005; Bove *et al.*, 2005). ABA inhibits embryo growth potential and endosperm cap weakening during coffee seed germination (da Silva *et al.*, 2004). A transient rise in ABA content in the embryo was evident early during imbibition. ABA treatment inhibits, and fluridone treatment accelerates, radicle protrusion of coffee seeds. Vegetation-derived ABA is also of ecological importance in the regulation of seed dormancy and germination. ABA leached from plant litter plays an important role in the germination control of the post-fire annual *Nicotiana attenuata* (Krock *et al.*, 2002; Preston *et al.*, 2002; Schwachtje and Baldwin, 2004).

Rupture of the testa and the endosperm are distinct and temporally separate events during the germination of *Nicotiana* seeds (Fig. 1; Leubner-Metzger, 2003b; Web site: 'The Seed Biology Place' http://www.seedbiology.de). Such two-step germination, with testa rupture subsequently followed by endosperm rupture, has also been demonstrated recently for *Arabidopsis* (Liu *et al.*, 2005; Müller *et al.*, 2005). The after-ripening-mediated promotion of tobacco germination is due to the promotion of both testa and subsequent endosperm rupture (Leubner-Metzger, 2005b). The addition of ABA to the medium during imbibition has effects resembling those of maternal ABA during seed development and residual ABA in mature seeds. It does not appreciably affect the kinetics of testa rupture, but it delays endosperm rupture and results in the formation of a novel structure, consisting of the enlarged radicle with a sheath of greatly elongated endosperm tissue (Leubner-Metzger, 2003b). The visible distinction between testa and endosperm rupture, and the finding that ABA inhibits endosperm rupture, but not testa rupture, is typical for the seed germination of the *Cestroideae* (*Nicotiana*, *Petunia*) subgroup of the *Solanaceae* (Krock *et al.*, 2002; Petruzzelli *et al.*, 2003a). A visible distinction between testa rupture and endosperm rupture is not possible for the seed germination of the *Solanoideae* (*Capsicum, Lycopersicon*) subgroup of the *Solanaceae* (Wu *et al.*, 2000; Leubner-Metzger, 2003b; Petruzzelli *et al.*, 2003a), nor the *Asteraceae* (*Lactuca*). However, ABA also inhibits the germination of *Solanoideae*-type seeds, and

experiments with the ABA-deficient *sit*^{w} mutant of tomato demonstrate that ABA deficiency is associated with a thinner testa (Hilhorst and Downie, 1995; Toorop *et al.*, 2000). In *Arabidopsis*, an arabinogalactan-protein (APG30) regulates the timing of germination by modulating ABA perception and presumably by altering the force generated by the penetrating radicle (Van Hengel and Roberts, 2003). Class I β-1,3-glucanase (βGlu I) is induced after testa rupture and just prior to endosperm rupture of *Nicotiana* seeds (Leubner-Metzger, 2003b). This induction is exclusively localized in the micropylar endosperm where the radicle will emerge. ABA inhibits the induction of βGlu I genes during tobacco seed germination and specifically delays endosperm rupture. Direct evidence for a causal role of βGlu I during endosperm rupture comes from sense-transformation of *N. tabacum* with a chimeric ABA-inducible βGlu I transgene (Leubner-Metzger and Meins, 2000). Sense-βGlu I transformation results in overexpression of βGlu I in seeds and promotes endosperm rupture of fresh, mature seeds and ABA-treated after-ripened seeds. βGlu I overexpression in the seed-covering layers can replace the promoting effect of after-ripening on testa rupture (Leubner-Metzger, 2002, 2005). ABA inhibition of germination and βGlu accumulation in the micropylar endosperm appears to be a widespread event during the germination of Solanaceous species (Wu *et al.*, 2000; Leubner-Metzger, 2003b; Petruzzelli *et al.*, 2003a).

Gibberellins (GA) release dormancy, promote germination and counteract ABA effects

According to the revised hormone-balance hypothesis for seed dormancy proposed by Karssen and Laçka (1986), ABA and GA act at different times and sites during 'seed life'. ABA induces dormancy during maturation, and GAs play a key role in dormancy release and in the promotion of germination. GA biosynthesis in developing seeds of many species leads to the accumulation and storage of either bioinactive GA precursors or bioactive GA (e.g. Groot *et al.*, 1987; Toyomasu *et al.*, 1998; Kamiya and Garcia-Martinez, 1999; Yamaguchi *et al.*, 2001). GA biosynthesis in developing seeds appears not to be involved in the establishment of primary dormancy *per se* (Karssen and Laçka, 1986; Groot *et al.*, 1987; Koornneef and Karssen, 1994; Bewley, 1997b), but in other aspects of seed development, including fertilization, embryo growth, assimilate uptake, fruit growth and the prevention of seed abortion, in tomato, pea and several species of the *Brassicaceae* (e.g. Groot *et al.*, 1987; Swain *et al.*, 1997; Batge *et al.*, 1999; Hays *et al.*, 2002; Koornneef *et al.*, 2002; Singh *et al.*, 2002). However, experiments with ABA-deficient and -insensitive mutants of maize, and with GA biosynthesis inhibitors, demonstrate that GA is a positive regulator of vivipary (White and Rivin, 2000; White *et al.*, 2000). Bioactive GAs accumulate prior to the ABA peak during embryo development of maize. Inhibition of GA biosynthesis mimics the effects of exogenous ABA, e.g. in suppressing vivipary. Interestingly, the GA/ABA ratio, and not the absolute hormone amounts, appears to control vivipary. Thus, it is possible that GA directly antagonizes ABA signalling during maize kernel development.

The temporal and spatial expression pattern of GA biosynthesis genes has been investigated during *Arabidopsis* seed germination (Yamaguchi *et al.*, 2001; Ogawa *et al.*, 2003; Yamauchi *et al.*, 2004). Bioactive GAs accumulate just prior to radicle protrusion and appear to occur in two separate locations within the embryo: (1) the early biosynthetic pathway, including the geranylgeranyl diphosphate cyclization reaction catalysed by *ent*-copalyl diphosphate synthetase (CPS), in the provascular tissue where *CPS1* gene promoter activity is localized, and (2) the late biosynthetic pathway, including the formation of bioactive GA by GA 3-oxidase, in the cortex and endodermis of the root, where *GA3ox1* and *GA3ox2* transcripts accumulate, and *GA3ox2* gene promoter activity is localized. This implies that intercellular transport of an intermediate of the GA biosynthetic pathway (probably *ent*-kaurene) is required to produce bioactive GA. Two functions for GA during seed germination have been proposed (reviewed by Hilhorst, 1995; Bewley, 1997a, b; Koornneef *et al.*, 2002; Leubner-Metzger, 2003b). First, GA increases the growth potential of the embryo. Secondly, GA is necessary to overcome the mechanical restraint conferred by the seed-covering layers, by weakening of the tissues surrounding the radicle. The localization of seed GA biosynthesis in the *Arabidopsis* radicle (Yamaguchi *et al.*, 2001) is consistent with the hypothesis that embryonic GA is released and triggers the weakening of seed-covering layers. This is further supported by the finding that at least some GA-responsive genes are expressed in non-GA-producing seed tissues (Ogawa *et al.*, 2003). Environmental cues like light and temperature can alter the tissue-specific localization of GA biosynthesis (Yamauchi *et al.*, 2004). The temporal and spatial pattern of GA biosynthesis and sensitivity are both important for the GA-mediated seed responses.

GA-deficient biosynthesis mutants (Table 1) of *Arabidopsis* (e.g. *ga1*) and tomato (e.g. *gib-1*) have been isolated (Karssen *et al.*, 1989; Hilhorst and Karssen, 1992; Koornneef and Karssen, 1994; Richards *et al.*, 2001). Seed germination of several of these GA-deficient mutants absolutely depends on the addition of GA to the medium during imbibition. The *Arabidopsis GA1* gene encodes a CPS, which catalyses a key cyclization step in early GA biosynthesis. It was

cloned by genomic subtraction based on a 5-kb deletion in the severe *ga1-3* mutant allele (Sun and Kamiya, 1994). The mechanisms imposing a GA requirement to promote the germination of dormant and non-dormant *Arabidopsis* seeds have been analysed using the GA-deficient mutant *ga1*, the ABA-deficient mutant *aba1*, and several testa mutants, which exhibit reduced seed dormancy (Debeaujon and Koornneef, 2000; Debeaujon *et al.*, 2000). Testa mutants are not resistant to GA biosynthesis inhibitors. However, in the presence of the inhibitors, or when transferred to a GA-deficient background, they are more sensitive to exogenous GA than the wild type. The germination capacity of the *ga1-1* mutant can be restored, without the help of exogenous GA, by removing the surrounding testa and endosperm, or by incorporating this mutation into a testa-mutant background. Debeaujon and Koornneef (2000) concluded that dormancy and germination are the net result of a balance between many promoting and inhibiting factors, including GA and ABA, which have the embryo and the testa as targets. Their results support the view that the GA requirement for dormancy release and germination is determined by: (1) ABA produced in the developing seeds and/or the state of dormancy set by ABA; and (2) the amount of ABA produced upon imbibition, especially in dormant seeds. Furthermore, when the restraint to radicle protrusion imposed by the seed envelopes is weakened by testa mutations, the embryo growth potential threshold required for germination is lowered. Thus, the GA requirement for *Arabidopsis* seed germination is determined by testa characteristics, embryonic growth potential and by embryonic ABA.

GA appears to regulate dormancy release and germination positively, by a complex interaction with ABA and environmental conditions. Regarding temperature as an environmental condition, Gonai *et al.* (2004) concluded that ABA is an important factor in the regulation of thermoinhibition of lettuce seed germination, and that GA affects the temperature responsiveness of the seeds through ABA metabolism. In members of the *Asteraceae* (e.g. lettuce), *Rubiaceae* (e.g. coffee) and *Solanaceae* (e.g. tomato, pepper and tobacco), the micropylar endosperm and testa tissues impose a constraint to radicle protrusion (Ni and Bradford, 1993; Hilhorst, 1995; Bewley, 1997a; Leubner-Metzger, 2003b; da Silva *et al.*, 2005). The assumption that the micropylar tissues impose a physical constraint to radicle protrusion is supported by puncture force measurements and surgical experiments. Removal of the micropylar testa and the endosperm tissues permits radicle growth under conditions that inhibit germination of intact seeds, e.g. of tobacco (Kincaid, 1935; Leubner-Metzger, 2003b), tomato (Liptay and Schopfer, 1983; Hilhorst, 1995) and lettuce (Bewley, 1997a; Dutta *et al.*, 1997). Since GA-deficient tomato *gib-1* mutant seeds germinate only upon treatment with GA, and isolated *gib-1* embryos elongate without a GA requirement, it can be concluded that the GA is needed to overcome the constraint of the seed coverings (Bassel *et al.*, 2004). In tomato and *Arabidopsis*, physiological, biochemical and genetic evidence suggests a role for GA in weakening the structures covering the embryo during germination (Bewley, 1997a; Yamaguchi and Kamiya, 2002; Yamauchi *et al.*, 2004). Weakening of the micropylar endosperm appears to be a prerequisite for germination and is likely to be achieved by cell-wall hydrolysis through the collaborative or successive action of several GA-induced cell-wall hydrolases (e.g. Bewley, 1997a; Nonogaki *et al.*, 2000; Leubner-Metzger, 2003b; Mo and Bewley, 2003; da Silva *et al.*, 2005). Endosperm rupture is inhibited by ABA, and endosperm weakening, at least in part and depending on the species, is also inhibited by ABA (Toorop *et al.*, 2000; Wu *et al.*, 2000; Leubner-Metzger, 2003b; da Silva *et al.*, 2004; Müller *et al.*, 2005). This direct or indirect GA–ABA antagonism is supported by physiological and biochemical experiments and by screens for suppressor mutants (Finkelstein *et al.*, 2002; Finkelstein, 2004). Several of the non-dormant *Arabidopsis* mutants that originated from a screen for seedling emergence in the presence of the GA biosynthesis inhibitor uniconazol are new alleles of *abi3* and *aba2* (Table 1, Fig. 2; Nambara *et al.*, 1992, 1998). Suppressor screens of the ABA-insensitive *abi1-1* mutation not only identified *sleepy1* (*sly1*), but also *ga1* mutant alleles (Table 1, Fig. 2; Steber *et al.*, 1998).

Among the GA-response mutants (Table 1) of *Arabidopsis*, the GA-insensitive *gai* mutant is characterized by a dwarf phenotype, increased GA content and complex seed effects that are consistent with a severely decreased GA sensitivity of dormancy release and germination (Derkx and Karssen, 1993; Koornneef and Karssen, 1994; Richards *et al.*, 2001). No appreciable seed germination of *gai* occurs in the dark, and only a combination of light with either chilling or dry after-ripening causes dormancy release and germination. The *Arabidopsis GAI* gene and its orthologues in other species encode nucleus-localized proteins that act as transcription factors, and appear to be negative regulators of the GA-signal transduction pathway. The GAI protein belongs to the DELLA subfamily of GRAS regulatory proteins, which includes several other negative regulators of GA responses, e.g. in *Arabidopsis RGA (repressor-of-ga1-3), RGL1 (RGA-like1), RGL2* and *RGL3* (Silverstone *et al.*, 1997; Richards *et al.*, 2001; Peng and Harberd, 2002). The DELLA domain region is involved in modulating the GA response, and its deletion in the *gai* mutant causes a gain-of-function mutation characterized by dominant GA-insensitive repression of GA responses.

In agreement with this, the loss-of-function allele *gai-t6* confers increased resistance of stem growth to the GA biosynthesis inhibitor paclobutrazol and wild-type-like germination in the light; *gai-t6* cannot rescue the non-germination phenotype of *ga1-3* (Dill and Sun, 2001; Richards *et al.*, 2001). The different negative DELLA-type regulators appear to possess separate, as well as overlapping, roles in GA responses (Fig. 2). It has been proposed that RGL1 plays a greater role in seed germination than do GAI and RGA (Wen and Chang, 2002), but RGL2 has been proposed to be the most important regulator of *Arabidopsis* seed germination in response to GA (Lee *et al.*, 2002; Tyler *et al.*, 2004; Cao *et al.*, 2005). Loss-of-function *rgl2* alleles suppress the GA-deficient seed germination phenotype conferred either by treatment with paclobutrazol or by *ga1-3*. Loss of function of the four *DELLA* genes (*RGL2, RGL1, RGA* and *GAI)* leads to light- and gibberellin-independent seed germination (Cao *et al.*, 2005). *Arabidopsis ga1-3* seeds lacking RGA + RGL1 + RGL2 or GAI + RGL1 + RGL2, confer GA-independent germination in the light but not in the darkness, while *ga1-3* seeds lacking GAI + RGA + RGL2 germinate both in the light and darkness (Cao *et al.*, 2005). This suggests that the destabilization or inactivation of RGA and GAI is not only triggered by GA, but also possibly by light. Therefore, DELLA proteins may act as integrators of environmental and endogenous cues to regulate seed germination.

RGL2 and the other DELLA-type proteins are transcription factors that inhibit GA responses and, thus, are considered to be negative regulators of GA signal transduction (Dill and Sun, 2001; Peng and Harberd, 2002). Therefore, GA should induce their degradation, as has been demonstrated for some DELLA-type regulators. Lee *et al.* (2002) found that *RGL2* transcripts are undetectable in dry *Arabidopsis* seeds and increase rapidly following seed imbibition; this expression is restricted to elongating regions of radicles. In contrast, Tyler *et al.* (2004) and Bassel *et al.* (2004) found that *RGL2* transcripts are abundant in dry *Arabidopsis* seeds, remain high during imbibition and germination, and decrease in emerged seedlings. An interpretative oversight of numerous *Arabidopsis* research papers is that early post-germination seedling growth is measured, and conclusions are extrapolated back to visible germination (radicle emergence), which occurs much earlier (Cohn, 1996). A careful time-course analysis of *Arabidopsis* seed germination showed that the *RGL2* mRNA decline occurred *after* radicle emergence, i.e. after germination had been completed (Bassel *et al.*, 2004). In tomato, there is only one *DELLA* gene (*LeGAI*); its mRNA is expressed during seed germination, during which there is an increase, but no decline, in its transcripts in either the embryo or in the endosperm (Bassel *et al.*, 2004). *LeGAI* mRNA contents also increase in GA-treated *gib-1* mutant seeds (endosperm and embryo) of tomato. There is also no decline of two *DELLA* gene transcripts in the radicle of soybean seeds before and after germination. Bassel *et al.* (2004) conclude that the embryo of soybean has the potential to complete germination without a requirement for GA biosynthesis or response. However, in seeds such as those of tomato and *Arabidopsis*, where germination is constrained by the surrounding structures, i.e. coat dormancy, an appropriate amount of GA is required to overcome this. But even when germination does require the intervention of GA, this is not achieved by the suppression of *DELLA* gene transcription. An assumption that is often made is that there is a direct correspondence between transcript and protein amounts. While this occurs in many cases, it is not always so, especially in seeds. Tyler *et al.* (2004) also investigated DELLA protein contents, while Lee *et al.* (2002) and Bassel *et al.* (2004, unfortunately their antibody did not work) base their conclusions solely on transcript amounts. Tyler *et al.* (2004) conclude from their experiments: 'We showed that RGL2 protein in imbibed seeds is rapidly degraded by GA treatment'. Although this might be the case, it is not supported by the data provided in the publication. No germination time-course is presented, and what is shown in their immunoblot analyses is that GA causes protein degradation of RGL2 and RGA at >50 h, i.e. in seedlings and not in seeds.

The *Arabidopsis sleepy1* (*sly1*) and the *comatose* (*cts*) mutants exhibit marked seed germination reduction that cannot be rescued by GA (Steber *et al.*, 1998; Russell *et al.*, 2000; Steber and McCourt, 2001; Strader *et al.*, 2004). The SLY1 and CTS proteins are proposed to be key factors involved in GA signalling of seeds. The *cts* mutant is impaired in seed dormancy release in the dry or imbibed state by after-ripening or cold treatment, respectively. *CTS* encodes a peroxisomal protein of the ATP-binding cassette (ABC) transporter class, and regulation of CTS function seems to be a major control point for the switch between dormancy and germination (Footitt *et al.*, 2002). CTS may be involved as an importer of precursors in the biosynthesis of jasmonic acid (Theodoulou *et al.*, 2005). Jasmonic acid inhibits germination of non-dormant seeds and affects dormancy (e.g. Bogatek *et al.*, 2002; Preston *et al.*, 2002). The *sly1* mutant was selected in a screen for suppressors of the ABA-insensitive *abi1-1* mutant. The *SLY1* gene is a positive regulator of GA signalling and an F-box protein (McGinnis *et al.*, 2003). RGA and RGL2 are substrates of SLY1, and this suggests that their degradation through the 26S proteasome pathway is involved in GA signalling. Tyler *et al.* (2004) show that the F-box protein SLY1 is required for RGL2 protein degradation in *Arabidopsis* seedlings. SLY1 has been postulated to be a 'key factor in GA reception' (Steber *et al.*, 1998;

McGinnis *et al.*, 2003). The F-box protein TIR1 is an auxin receptor that mediates auxin responses by promoting the degradation of the Aux/IAA transcriptional repressors (Dharmasiri *et al.*, 2005). We, therefore, postulate that the F-box protein SLY1 is a GA receptor that mediates GA responses by promoting the degradation of DELLA-type transcription repressors, e.g. RGL2, in seeds and seedlings.

The constitutive GA-response mutant *spindly* (*spy*) of *Arabidopsis* has been isolated in a screen for seeds able to germinate in the presence of paclobutrazol (Izhaki *et al.*, 2001; Richards *et al.*, 2001; Swain *et al.*, 2001). Thus, the GA requirement for seed dormancy release and germination is decreased in the *spy* mutant. The elongated growth phenotype of *spy* mutant plants resembles wild-type plants treated with GA. Furthermore, *spy* mutations suppress the effects of GA deficiency on germination, and overexpression of (wild-type) *SPY* inhibits seed germination of *Arabidopsis* and petunia. The *Arabidopsis SPY* gene and its orthologues in other species encode tetratricopeptide repeat proteins that might function as *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) transferases (Hartweck *et al.*, 2002). SPY appears to act as a negative regulator of GA responses upstream of GAI. A possible function of SPY is to regulate GAI function by *O*-GlcNAc modification and thereby influence the nuclear localization of GAI (Richards *et al.*, 2001).

The release from photodormancy and promotion of germination of light-requiring seeds of many species are regulated by phytochrome (Kamiya and Garcia-Martinez, 1999; Yamaguchi and Kamiya, 2002). Red light up-regulates the biosynthesis of bioactive GA_1 and GA_4 by inducing GA biosynthetic genes in germinating seeds of lettuce and *Arabidopsis* (Toyomasu *et al.*, 1993, 1998; Yamaguchi *et al.*, 1998, 2001; Ogawa *et al.*, 2003). Gene induction of GA 3-oxidases (i.e. GA 3β-hydroxylases) during imbibition is controlled by light via phytochrome. In lettuce, synthesis of a GA-degrading enzyme (GA 2-oxidase, *Ls*GA2*ox2*) is suppressed by red light, which might lead to an additional increase in bioactive GA in these seeds (Nakaminami *et al.*, 2003). Light-induced GA biosynthesis during *Arabidopsis* seed germination appears to occur in two separate embryo tissues, the provascular tissue (early steps, CPS) and in the cortex and endodermis of the radicle (late steps, GA 3-oxidase transcripts *GA3ox1* and *GA3ox2*); the intercellular transport of a GA precursor has been proposed (Yamaguchi *et al.*, 2001; Ogawa *et al.*, 2003). Stratification (cold treatment at 4 °C), commonly used to promote and synchronize seed germination, caused *GA3ox1* mRNA expression in the entire radicle and in the aleurone layer, and an increase in bioactive GA content (Yamauchi *et al.*, 2004). Thus, light and low temperature can modulate the spatial expression pattern of GA biosynthetic genes. *N. tabacum* seed germination is also regulated by phytochrome, and GA substitutes for the red-light trigger needed to release photodormancy, inducing dark germination (Kretsch *et al.*, 1995; Leubner-Metzger, 2003b). Similar results have been obtained for *N. attenuata* seeds, where GA treatment causes germination in the dark (Schwachtje and Baldwin, 2004). Normally, those seeds require smoke for breaking their dormancy, but smoke treatment in the dark without addition of GA is not sufficient to cause germination. Therefore, smoke exposure may increase the sensitivity of *N. attenuata* seeds to GA. Far less is known about the role of GA sensitivity during the after-ripening-mediated release of *N. tabacum* photodormancy. Fresh *N. tabacum* seeds are photodormant, i.e. they do not germinate during incubation in darkness, and even prolonged incubation in the dark does not induce testa rupture, βGlu I accumulation or endosperm rupture (Leubner-Metzger, 2002, 2003b). After-ripening contributes to the release of photodormancy, but its effect varies greatly between different seed batches, as reported for several tobacco cultivars. The GA requirements for photodormancy release of fresh and completely after-ripened photodormant seed batches are equal.

Non-photodormant tobacco seeds have lost the GA requirement for dark germination, which could be due to increased GA sensitivity and/or increased endogenous GA (Leubner-Metzger, 2001, 2002, 2005). GA treatment of dark-imbibed non-photodormant tobacco seeds increases the rate of germination in the dark, demonstrating that GA is also a positive regulator of germination speed. Thus, GA not only releases dormancy, it promotes germination rate and onset, as also supported by other studies with GA biosynthesis inhibitors and with non-dormant seeds (Steinbach *et al.*, 1997; Tadeo *et al.*, 1997; Kamiya and Garcia-Martinez, 1999; Yamaguchi and Kamiya, 2002). Endogenous GAs are required for embryo cell elongation and endosperm cap weakening (e.g. Bewley, 1997a; da Silva *et al.*, 2005). Taken together, GA releases coat and embryo dormancy, promotes germination and counteracts inhibitory ABA effects, directly or indirectly.

Ethylene promotes seed germination and counteracts ABA effects

Ethylene is implicated in the promotion of germination of non-dormant seeds of many species (Corbineau *et al.*, 1990; Esashi, 1991; Kepczynski and Kepczynska, 1997; Matilla, 2000). In some species (e.g. peanut, sunflower), ethylene releases dormancy. In many species, ethylene alone is not sufficient to release seed dormancy, even when it promotes germination of non-dormant seeds of a given species.

For example, inhibition of ethylene biosynthesis or action inhibits germination in the light, but treatment with ethylene does not release photodormancy and permit subsequent germination in darkness (e.g. Saini *et al.*, 1989; Leubner-Metzger *et al.*, 1998). Increased ethylene evolution is associated with the germination of eudicot seeds, e.g. *Cicer arietinum* (Matilla, 2000; Gómez-Jiménez *et al.*, 2001), *Pisum sativum* (Gorecki *et al.*, 1991; Petruzzelli *et al.*, 1995), *L. esculentum* (Lashbrook *et al.*, 1998), *N. tabacum* (Leubner-Metzger *et al.*, 1998), and *Lactuca sativa* (Saini *et al.*, 1989; Matilla, 2000). The need for endogenous ethylene for optimal germination of non-dormant seeds has been demonstrated in several of these studies by utilizing the ethylene action inhibitors 2,5-norbornadiene (NBD) or 1-methylcyclopropene (1-MCP) (Sisler and Serek, 2003). Ethylene production is higher in non-dormant compared to dormant seeds (Esashi, 1991; Kepczynski and Kepczynska, 1997; Matilla, 2000), and is positively correlated with the germination rate of non-dormant pea seeds (Gorecki *et al.*, 1991). A major peak of ethylene evolution coincides with the completion of germination, but ethylene production is already detectable very early during imbibition and before radicle protrusion through the covering layers.

Several hypotheses have been proposed to explain the mechanism(s) of ethylene action in germinating seeds (Esashi, 1991; Kepczynski and Kepczynska, 1997; Matilla, 2000). The primary action of ethylene could be the promotion of radial cell expansion in the embryonic hypocotyl, increased seed respiration or increased water potential. High-level induction of ABA-sensitive class I β-1,3-glucanase (βGlu I) gene expression in the micropylar endosperm of tobacco requires endogenous ethylene and promotes endosperm rupture (Leubner-Metzger *et al.*, 1998). The molecular mechanisms of gene regulation by ethylene have been thoroughly studied in vegetative tissues, during flower senescence and fruit ripening (Hall *et al.*, 2001; Klee, 2004; Stepanova and Alonso, 2005). However, there are also a few reports for ethylene-altered gene expression during seed germination: three members of the *ETR* gene family encoding putative ethylene receptors in tomato (Lashbrook *et al.*, 1998), cysteine proteinase in chick-pea (Cervantes *et al.*, 1994), βGlu I in tobacco and pea (Leubner-Metzger *et al.*, 1998; Petruzzelli *et al.*, 1999), and 1-aminocyclopropane-1-carboxylic acid (ACC, the ethylene precursor) oxidase (ACO, the ethylene-forming enzyme) of pea (Petruzzelli *et al.*, 2000, 2003b) and of *Brassica rapa* (Puga-Hermida *et al.*, 2003).

Ethylene is perceived by a family of receptors related to ETR1 of *Arabidopsis*, and ethylene binding inhibits the signalling activities of these receptors (Hall *et al.*, 2001; Klee, 2004; Stepanova and Alonso, 2005). An increase in responsiveness to ethylene is associated with the germination of non-endospermic and endospermic seeds, e.g. pea (Petruzzelli *et al.*, 2000) and tomato (Lashbrook *et al.*, 1998). The transcript amounts of three tomato ethylene receptor genes increase during seed germination. Ethylene biosynthesis and sensitivity are both important for the germination of tobacco (Leubner-Metzger *et al.*, 1998) and *Arabidopsis* (Beaudoin *et al.*, 2000; Ghassemian *et al.*, 2000; Gallardo *et al.*, 2002a, b; Siriwitayawan *et al.*, 2003). ABA inhibits the seed germination of *Arabidopsis*, an effect that can be partially reversed by treatment with ACC (Ghassemian *et al.*, 2000). The *etr1* mutant seed of *Arabidopsis* exhibits poor germination (Bleecker *et al.*, 1988; Chiwocha *et al.*, 2005), and the seeds are also hypersensitive to ABA (Beaudoin *et al.*, 2000). In the absence of ethylene, the ETR1 activates CTR1, which is a negative regulator of downstream signalling components. CTR1 is inactive in the presence of ethylene. Etiolated seedlings of the *constitutive triple response1* (*ctr1*) mutant of *Arabidopsis* (Table 1, Fig. 2) are characterized by a constitutive ethylene triple response, i.e. inhibition of shoot elongation, shoot swelling and intensified curvature of the apical hook. Seed germination of *ctr1* is less sensitive to ABA, and freshly harvested *ctr1* seeds germinate slightly faster than wild-type seeds (Beaudoin *et al.*, 2000). CTR1 functions as a RAS-like mitogen-activated protein kinase kinase kinase (MAPKKK), and is the start of an ethylene-activated MAPK pathway (Chang, 2003; Ouaked *et al.*, 2003). Additional ethylene signalling pathways exist, and an ethylene receptor complex is proposed to consist of ETR1, CTR1 and phosphorelay intermediates (Hall *et al.*, 2001; Lohrmann and Harter, 2002). This type of phosphorelay signalling, which is likely to function via histidine protein kinases, involves signal receptors for ethylene, cytokinins, light and osmolarity. The phosphorelay permits the conversion of hormonal and environmental signals into biochemical reactions, allows the linking of the different signals to form a complex signalling network and provides signal integration.

EIN2 is a downstream signalling component that, in the absence of ethylene, is down-regulated by CTR1. As with *etr1*, the *ethylene insensitive2* (*ein2*) mutants of *Arabidopsis* are characterized by higher seed dormancy and by hypersensitivity to ABA (Table 1; Fig. 2). Interestingly, *ctr1* and *ein2* mutants have been recovered as enhancer and suppressor mutants, respectively, of the ABA-insensitive seed germination phenotype of the *abi1-1* mutant (Beaudoin *et al.*, 2000). *Arabidopsis enhanced response to ABA3* (*era3*) mutants are characterized by increased sensitivity of the seed to ABA and by overaccumulation of ABA (Ghassemian *et al.*, 2000). The *era3* alleles turned out to be new alleles of the *EIN2* locus (Fig. 2). The *ein2-45* allele increases seed dormancy, but this effect is completely counteracted by severely ABA-insensitive mutations,

such as *abi3-4* (Beaudoin *et al*., 2000). As a consequence, the *ein2-45 abi3-4* double mutant is as non-dormant as the *abi3-4* single mutant. The non-dormant phenotype of the *ein2 abi3-4* double mutant suggests that ethylene suppresses seed dormancy by inhibiting ABA action. Thus, EIN2 is a possible negative regulator of ABA response. However, as the *abi3-4* allele causes vivipary, pleiotropic effects may mask real interactions. The enhanced dormancy of *etr1* and *ein2* seeds suggests that endogenous ethylene is a negative regulator of *Arabidopsis* seed dormancy. The finding that *ein2* mutations can suppress, and *ctr1* mutations can enhance, the ABA-insensitive germination phenotype of *abi1* suggests that ethylene also influences the sensitivity of the seed to ABA. These results indicate a strong interaction between the ethylene and ABA signal transduction pathways, and a key conclusion is that ethylene can promote germination by directly interfering with ABA signalling (Beaudoin *et al.*, 2000; Ghassemian *et al*., 2000). ABA–ethylene interactions are also evident in seeds and seedlings of other species (e.g. Leubner-Metzger *et al.*, 1998; Spollen *et al.*, 2000). In summary, ethylene alone probably cannot act as a positive regulator of germination, but can only act by interfering with ABA signalling.

Genetically downstream of EIN2 are transcription factors that regulate gene expression in response to ethylene (Fig. 2). Ethylene–ABA interactions are also manifested at this level of ethylene signalling (Ghassemian *et al.*, 2000). EIN3 and EIN3-LIKE proteins bind to the promoter of the *Arabidopsis ethylene responsive factor 1* (*ERF1*) gene, which encodes an ethylene-responsive element binding protein (EREBP)-type transcription factor, and thereby confers a hierarchy of transcription factors involved in ethylene signalling (Solano *et al.*, 1998; Lee and Kim, 2003). The EREBP-type transcription factors mediate ethylene regulation of gene expression (Figs 1, 2). They bind to the GCC box within the positively acting ethylene-responsive element (ERE) of target promoters, and this *cis*-regulatory element is necessary and sufficient for the regulation of transcription by ethylene. Ethylene is involved in endosperm rupture and high βGlu I expression during tobacco seed germination, but it does not affect the spatial and temporal pattern of βGlu I expression (Leubner-Metzger *et al.*, 1998). A promoter deletion analysis in germinating tobacco seeds suggests that the distal region, which contains the ERE, is required for high, ethylene-sensitive expression; the proximal region is necessary and sufficient for low-level micropylar-endosperm specific expression; and both regions contribute to down-regulation by ABA. Transcription factors, like the tobacco EREBP-3 (Leubner-Metzger *et al.*, 1998), the *Arabidopsis* bromodomain protein IMB1 (Duque and Chua, 2003) and the tobacco EIN3-LIKE homologues (Lee and Kim, 2003), are involved in mediating ABA–ethylene regulation of β-1,3-glucanase expression. Transcripts of EREBP-3 and IMB1 are stored in air-dry seeds, induced during early seed imbibition, down-regulated during late germination and regulated by ABA (Leubner-Metzger *et al.*, 1998; Duque and Chua, 2003). Interestingly, sequences that are homologous to the GCC box are also present in the promoters of some ACO genes (see below, and references in Petruzzelli *et al*., 2000).

Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of ACC oxidase (ACO), which catalyses the final step of ethylene biosynthesis (Petruzzelli *et al.*, 2000, 2003b). An early onset and sequential induction of ACC biosynthesis, *Ps-ACO1* mRNA, a 36-kDa Ps-ACO1 protein, ACO activity and ethylene production are localized almost exclusively in the embryonic axis. Within the axis, ethylene biosynthesis and responsiveness are localized in the cell elongation and differentiation zones of the radicle. Maximal contents of ACC, Ps-ACO1 and ethylene evolution occur when radicle emergence is just completed. Accumulation of *Ps-ACO1* mRNA, protein and ACO enzyme activity in the embryonic axis during late germination requires ethylene, whereas *Ps-ACS1* mRNA and overall ACC contents are not induced by ethylene. Ethylene does not induce ACO in the embryonic axis during early germination. Similar observations have been made for the highly homologous ACO in chickpea seeds (Nicolas *et al.*, 1998; Matilla, 2000; Gómez-Jiménez *et al.*, 2001). ABA inhibits chickpea ACO expression, ACC accumulation and ethylene production during germination, but not after radicle emergence. This suggests that interference of ethylene and ABA signalling confers an opposing feedback regulation of ethylene production in the embryonic axis. Calcium is required for ethylene and ABA responses during pea and chickpea seed germination (Nicolas *et al.*, 1998; Petruzzelli *et al.*, 2003b). Although ABA and ethylene act antagonistically on seed germination, they both inhibit root elongation of *Arabidopsis* seedlings (Beaudoin *et al.*, 2000; Ghassemian *et al.*, 2000; Petruzzelli *et al.*, 2003b). Thus, seeds and vegetative tissues differ with regard to the ethylene–ABA interactions and integrated responses.

Cross-talk between ethylene and GA during the transition from seed dormancy to germination has been postulated from the hormonal regulation of a GA 20-oxidase in *Fagus sylvatica* (Calvo *et al.*, 2004). GA treatment induces *ACO* expression in imbibed *ga1-3* seeds (Ogawa *et al.*, 2003). An excess of ethylene can bypass the GA requirement and induce germination of *Arabidopsis ga1* mutant seeds imbibed in the light, the effect being much weaker in darkness (Karssen *et al.*, 1989; Koornneef and Karssen, 1994). The GA–ethylene interaction seems reciprocal, because high

GA concentrations restore the germination of *etr1* mutant seeds to wild-type levels (Bleecker *et al.*, 1988). By contrast, ethylene does not break dormancy of the tomato *gib-1* mutant, although it promotes the germination of tomato wild-type seeds (Nelson and Sharples, 1980; Groot and Karssen, 1987). Treatments with GA, ethylene or cytokinins alone are not able to overcome lettuce thermoinhibition in the dark (Saini *et al.*, 1989; Matilla, 2000). In addition to exogenous ethylene, at least one other hormone, or light, is required. Ethylene biosynthesis is essential for the release of thermoinhibition in the dark following application of GA or cytokinin, as well as for the light-induced release of thermoinhibition. The cytokinin or ethylene requirements for overcoming thermoinhibition of lettuce germination can also be eliminated or diminished by removing or weakening the endosperm. Thus, ethylene biosynthesis is involved as a negative regulator of coat dormancy. Furthermore, thermoinhibition of lettuce is correlated with the accumulation of ABA (Yoshioka *et al.*, 1998).

Taken together, promotion of seed germination by ethylene appears to be a general phenomenon, while in some species ethylene also appears to be required for dormancy release. Ethylene seems to counteract the inhibitory effects of ABA on seed germination by interfering with ABA signalling.

Brassinosteroids promote seed germination

Brassinosteroids (BR) and GA interact with light in regulating elongation of shoots and photomorphogenesis of seedlings by apparently independent pathways (Altmann, 1999; Szekeres, 2003). Endogenous BR have been identified in seeds of several species (e.g. Adam and Marquardt, 1986; Schmidt *et al.*, 1997). They are perceived by the plasma membrane-localized leucine-rich repeat receptor kinase, BRI1 (BR insensitive 1) in *Arabidopsis*, and several homologues have been identified utilizing BR-insensitive mutants of other species (Szekeres, 2003; Kinoshita *et al.*, 2005). Whereas BR application enhances germination of certain parasitic angiosperms (Takeuchi *et al.*, 1991, 1995), cereals (Yamaguchi *et al.*, 1987), *Arabidopsis* mutants (Steber and McCourt, 2001) and tobacco (Leubner-Metzger, 2001), it does not affect the germination of non-photodormant, non-endospermic cress seeds imbibed in the dark (Jones-Held *et al.*, 1996). Germination of the endospermic seeds of parasitic *Orobanche* and *Striga* species is, in contrast to *Arabidopsis* and tobacco, inhibited by light (Takeuchi *et al.*, 1991, 1995; Rugutt *et al.*, 2003). BR, ethylene and GA cannot substitute for the conditioning treatment with strigol, which is needed for inducing germination of unconditioned (i.e. dormant) seeds of those species. Conditioning removes the restriction on the ethylene biosynthetic pathway and increases the capacity to produce ethylene (Babiker *et al.*, 2000). In *Striga hermothica* the transcripts of ACC synthase and ACO increase after treatment with the strigolactone GR24 and after conditioning, supporting the idea that endogenous ethylene is involved in the germination of *Striga* seeds (Sugimoto *et al.*, 2003). ACO enzyme activity of *Striga asiatica* seeds can only be stimulated by conditioning, but not by strigol treatment (Mohamed *et al.*, 2001). Germination of conditioned (i.e. non-dormant) *Orobanche* and *Striga* seeds imbibed in the light and in the dark is promoted by treatment with BR. ABA inhibits germination of *Orobanche* seeds. Consistent with this, inhibition of carotenoid biosynthesis by norflurazon or fluridone promotes strigol-induced germination of *Orobanche* seeds. Unexpectedly, this effect is not due to a decreased ABA content of the seeds and therefore must be attributed to perturbations other than inhibition of ABA biosynthesis (Chae *et al.*, 2004).

BR promotes the germination of pre-chilled (i.e. non-dormant) seeds of the BR-deficient biosynthesis mutant *det2-1* and the BR-insensitive response mutant *bri1-1* of *Arabidopsis* (Table 1) imbibed in the light (Steber and McCourt, 2001). Seed germination of *det2-1* and *bri1-1* is more strongly inhibited by ABA than is germination of the wild type, and therefore BR is able to partially overcome the inhibition of germination by ABA. BR treatment rescues the germination phenotype of the severe GA-deficient biosynthesis mutant *ga1-3*, which normally requires GA treatment for dormancy release and germination. BR treatment also partially rescues the germination phenotype of the severe GA-insensitive response mutant *sly1*, which also cannot be rescued by treatment with GA. Interestingly, a new allele for *sly1* has been identified in a screen for BR-dependent germination that suggests interactions between BR and GA signalling in seeds (Steber *et al.*, 1998; Steber and McCourt, 2001). These results point to a role for BR in stimulating germination of *Arabidopsis* seeds. This is further supported by the germination phenotype of the *gpa1* mutant of *Arabidopsis* (Ullah *et al.*, 2002). The *GPA1* gene encodes the α-subunit of a heterotrimeric G protein. Seeds with the *gpa1* null mutation are 100-fold less responsive to GA, and GPA1 overexpressing seeds are hypersensitive to GA. The *gpa1* mutant seeds are also completely insensitive to BR rescue of germination when the seed GA content is reduced. These findings support the view that there is a complex interaction between GA and BR in regulating seed germination of *Arabidopsis*.

BR promotes seedling elongation and germination of non-photodormant tobacco seeds, but does not appreciably affect testa rupture and the subsequent induction of βGlu I in the micropylar endosperm (Leubner-Metzger, 2001). Treatment with BR, but not

GA, accelerates endosperm rupture of tobacco seeds imbibed in the light. In contrast, in dark-imbibed seeds, both BR and GA promote endosperm rupture, but only GA enhances βGlu I induction. Promotion of endosperm rupture by BR is dose-dependent, and 0.01 μM brassinolide is most effective. BR and GA promote ABA-inhibited dark-germination of non-photodormant seeds, but only GA replaces light in inducing βGlu I. These results indicate that BR and GA promote tobacco seed germination by distinct signal transduction pathways and distinct mechanisms. GA and light act in a common pathway to release photodormancy, whereas BR does not release photodormancy. βGlu I induction in the micropylar endosperm and release of coat dormancy appear to be associated with the GA/light pathway, but not with BR signalling. Xyloglucan endo-transglycosylase (XET) enzyme activity accumulates in the embryo and the endosperm of germinating tobacco seeds, and this appears to be partially controlled by BR (Leubner-Metzger, 2003a). A GA-regulated XET mRNA also is expressed exclusively in the micropylar endosperm of tomato seeds (Chen *et al.*, 2002). Therefore, it is possible that the XET induction in the tobacco embryo is controlled by BR. These findings suggest as a model for the endosperm-limited germination of tobacco: (1) Photodormancy is released exclusively by the GA/light pathway. (2) Promotion of subsequent endosperm rupture by the BR and the GA/light signal transduction pathways is achieved by independent and distinct mechanisms. (3) ABA inhibits endosperm rupture by interfering with both pathways. (4) The GA/light pathway regulates βGlu I induction in the micropylar endosperm and controls endosperm weakening. (5) The BR pathway promotes endosperm rupture of non-dormant seeds, at least in part, by enhancing the growth potential of the embryo (Leubner-Metzger, 2001, 2003b).

Taken together, these findings suggest that GA and BR act in parallel to promote cell elongation and germination and to counteract the inhibitory action of ABA. Since BR stimulates germination of the GA-insensitive mutant *sly1*, it is unlikely that BR acts by increasing GA sensitivity. It is possible that BR acts by stimulating GA biosynthesis in *Arabidopsis* seeds imbibed in the light (Steber and McCourt, 2001). However, this stimulation is unlikely to occur in tobacco, because BR does not promote the expression of βGlu I, which is induced by GA in the dark (Leubner-Metzger, 2001). It is known that BR can stimulate ethylene production, and ethylene treatment can rescue the germination phenotype of the GA-deficient *Arabidopsis ga1-1* mutant (Karssen *et al.*, 1989; Koornneef and Karssen, 1994; Steber and McCourt, 2001). However, there are several arguments against the hypothesis that BR acts via ethylene: (1) Ethylene does not increase in cress seedlings following BR treatment of seeds (Jones-Held *et al.*, 1996). (2) Endogenous ethylene promotes βGlu I accumulation in the micropylar endosperm of tobacco, but BR treatment promotes endosperm rupture without enhancing βGlu I accumulation (Leubner-Metzger *et al.*, 1998; Leubner-Metzger, 2001). (3) Ethylene rescue of *ga1-1* seed germination results in seedlings exhibiting a triple response, but BR rescue of *ga1-3* seed germination results in seedlings that do not (Steber and McCourt, 2001). Another possibility is that BR action occurs via auxin, because auxin response factors are subject to BR regulation (Müessig *et al.*, 2003). If BR promotes germination via embryo expansion, this effect is probably specific to embryos and is not found in seedlings.

Cytokinins and auxins

Cytokinins are present in developing seeds and accumulate predominantly in the liquid endosperm (Emery *et al.*, 2000; Fischer-Iglesias and Neuhaus, 2001; Mok and Mok, 2001). It has been proposed that the endosperm is a source of cytokinins needed for the promotion of cell division in the embryo. They may have roles in embryogenesis, in embryonic pattern formation, in the early period of grain filling of cereals, and in enhancing sink strength. In sorghum grains the cytokinin content is high in the embryo, low in endosperm, and it declines during imbibition (Dewar *et al.*, 1998). After radicle protrusion a cytokinin peak is associated with α-amylase accumulation. This cytokinin might play a role in cell division and elongation of the emerged root. It may also be redistributed within the embryo to regions where it effectively concentrates and directs root growth. There is a high ABA content in the embryo during germination, very low amounts of bioactive GA, and it has been proposed that the cytokinin/ABA interaction plays a role in controlling *Sorghum* germination (Dewar *et al.*, 1998). Confirmation of these trends in other species may revive interest in the concept of a permissive role of cytokinins in the regulation of germination, as developed by Khan (1975). For example, the ability of cytokinins alone (GA is without effect) to overcome ABA-inhibited germination of isolated Grand Rapids lettuce embryos (Bewley and Fountain, 1972) merits reinvestigation at the molecular level. It would not be surprising to learn that there is extensive cross-talk between cytokinins and other plant hormone signalling systems in the regulation of dormancy and germination.

Many species are known where cytokinins alone break seed dormancy (summarized in Cohn and Butera, 1982). During the conditioning of parasitic *Orobanche* and *Striga* species and the release of lettuce thermoinhibition, cytokinins appear to contribute to

the promotion of dormancy release and subsequent germination by enhancing ethylene biosynthesis (Saini *et al.*, 1989; Babiker *et al.*, 2000; Matilla, 2000). A cytokinin–ethylene connection is also supported by the discovery that the *Arabidopsis cytokinin-resistant1* (*ckr1*) mutant is insensitive to ethylene and allelic to the ethylene-insensitive mutant *ein2* (Fischer-Iglesias and Neuhaus, 2001). Cytokinin-resistant mutants of *N. plumbaginifolia* have been isolated that exhibit reduced seed dormancy and pleiotropic seed effects suggestive of cytokinin–ABA interactions (Rousselin *et al.*, 1992).

Auxins seem to play a major role in embryogenesis, providing positional information for the coordination of correct cellular patterning from the globular stage onwards (Fischer-Iglesias and Neuhaus, 2001; Teale *et al.*, 2005). While recent molecular and genetic data support an essential role for auxins in apical–basal pattern formation during embryogenesis, very little is known at the molecular level about the role of auxin during seed germination. Free indoleacetic acid (IAA) decreases during the imbibition of *Sorghum* grains (Dewar *et al.*, 1998), and auxin regulates catalase expression in the scutellum of germinating maize kernels (Guan and Scandalios, 2002). A relationship between IAA, dormancy and preharvest sprouting of wheat has been reported (Ramaih *et al.*, 2003).

IAA is released from conjugates stored in seeds of Scots pine during germination (Ljung *et al.*, 2001). A peak of free IAA occurs prior to the initiation of root elongation and coincides with initial seed swelling during imbibition. In bean seeds, increasing synthesis of IAA occurs during germination (Bialek *et al.*, 1992). Free IAA contents decline during radicle emergence, and new IAA synthesis is established in the emerged seedling. An IAA-modified protein from bean seeds, lAP1, is associated with rapid growth during seed development (Walz *et al.*, 2002). Moreover, this protein undergoes rapid degradation during germination. IAA-modified proteins represent a distinct class of conjugated phytohormones and appear to be the major form of auxin in bean seeds. The post-germination accumulation of ACO and ethylene production of chick-pea seedlings is promoted by IAA (Gómez-Jiménez *et al.*, 2001). IAA has no appreciable effect on ACO expression prior to or during germination. Several auxin-resistant mutants of *N. plumbaginifolia* have been isolated that exhibit reduced seed dormancy, and pleiotropic effects suggest auxin–GA interactions (Rousselin *et al.*, 1992). However, there appears to be no auxin effect on the seed germination of GA-deficient *Arabidopsis* and tomato mutants (Koornneef and Karssen, 1994).

Whether or not auxins play a role in *Arabidopsis* germination is unclear; however, exogenous GA up-regulates the early expression of several auxin-related genes (Ogawa *et al.*, 2003) that putatively encode auxin carrier proteins, as well as genes that may be associated with auxin biosynthesis. This suggests that GA causes substantial changes in auxin content and transport during *Arabidopsis* seed germination.

Summary and conclusions

Dormancy and germination are complex phenomena that are controlled by a large number of genes, which are affected by both developmental and environmental factors (Bewley, 1997b; Koornneef *et al.*, 2002). We are only beginning to understand part of the intensive cross-talk between the hormones implicated in these processes (Brady and McCourt, 2003; Finkelstein, 2004). A crucial role for ABA has been identified in inducing seed dormancy. Factors determining spatial and temporal ABA content and sensitivity patterns in seeds positively regulate induction of dormancy and probably its maintenance, and negatively regulate dormancy release and germination. GA releases dormancy, promotes germination and counteracts inhibitory ABA effects, directly or indirectly. GA is required for embryo cell elongation, for overcoming coat restrictions to germination of non-dormant and dormant seeds, and for inducing endosperm weakening. BR and ethylene also counteract the inhibitory effects of ABA on seed germination, but in most species they appear to act after dormancy has been released by GA. Ethylene seems to counteract the inhibitory effects of ABA on seed germination by interfering with ABA signalling. This is completely different from the situation in seedling root growth, which is inhibited by both hormones. The ABA–ethylene antagonism in seeds shows that hormonal interactions and responses differ between germinating seeds and vegetative tissues.

Molecular approaches using the angiosperm model species, *Arabidopsis thaliana,* have provided valuable data sets from transcriptome and proteome approaches. An absolute requirement for this type of evaluation is that it must be based on solid seed physiology. Cohn (1996) has discussed many of the relevant issues; additionally, numerous, more recent studies indicate that mutant seeds must be compared to corresponding wild-type seeds harvested from plants grown simultaneously under the same conditions. A good example of a 'solid-physiology' proteome approach is the work of Rajjou *et al.* (2004, http://seed.proteome.free.fr); it is based on germination time courses, in which germination *per se*, i.e. radicle emergence, was counted.

Exploiting natural variation and using clearly dormant *Arabidopsis* ecotypes, like Cvi, are promising areas for seed dormancy research (Koornneef *et al.*, 2002). However, the time has also come to move beyond *Arabidopsis* and apply molecular approaches

like transcriptomics and proteomics to other seed model species. Seeds are an important factor of the enormous angiosperm biodiversity (Baskin and Baskin, 2004). The comparative investigation of representatives from each phylogenetic angiosperm clade offers the opportunity for seed research that enhances our understanding of the evolution of biodiversity.

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