Journal of Experimental Botany, Vol. 52, No. 362, pp. 1753–1759, September 2001

Journal of Experimental Botany

Antisense-transformation reveals novel roles for class I β-1,3-glucanase in tobacco seed after-ripening and photodormancy

Gerhard Leubner-Metzger[1,2,3] and Frederick Meins Jr[1]

[1] *Friedrich Miescher-Institute, Maulbeerstrasse 66, CH-4058 Basel, Switzerland*

[2] *Institut für Biologie II, Albert-Ludwigs-Universität, Schänzlestr. 1, D-79104 Freiburg, Germany*

Received 27 September 2000; Accepted 17 May 2001

Abstract

Little is known about the molecular basis for seed dormancy, after-ripening, and radicle emergence through the covering layers during germination. In tobacco, endosperm rupture occurs after testa rupture and is the limiting step in seed germination. Class I β-1,3-glucanase (βGLU I), which is induced in the micropylar endosperm just prior to its penetration by the radicle, is believed to help weaken the endosperm wall. Evidence is pesented here for a second site of βGLU I action during after-ripening. Tobacco plants were transformed with antisense βGLU I constructs with promoters thought to direct endosperm-specific expression. Unexpectedly, these transformants were unaffected in endosperm rupture and did not exhibit reduced βGLU I expression during germination. Nevertheless, antisense βGLU I transformation delayed the onset of testa rupture in light-imbibed, after-ripened seeds and inhibited the after-ripening-mediated release of photodormancy. It is proposed that βGLU I expression in the dry seed contributes to the after-ripening-mediated release of seed dormancy.

Key words: Abscisic acid, after-ripening, coat-enhanced seed dormancy, endosperm-limited seed germination, *β*-1,3-glucanase, sense- and antisense transformation.

Introduction

Seed development is completed by a period of maturation when water content decreases, abscisic acid (ABA) accumulates, and primary dormancy is established. ABA is involved in the induction and also in the maintenance of the dormant state, a block of an intact viable seed to complete germination under favourable conditions (Li and Foley, 1997; Bewley, 1997*a*; Grappin *et al*., 2000). After-ripening of freshly harvested, mature seeds under warm, dry conditions for several months alters the primary dormancy of many species, including tobacco (Kasperbauer, 1968; Koornneef and Karssen, 1994; Hilhorst, 1995; Leubner-Metzger and Meins, 2000). Germination starts with imbibition of water by the dry seed and visible protrusion of the radicle through the ruptured covering layers is generally considered as completion of germination. Little is known about the molecular basis for after-ripening and the rupture and penetration of covering layers during the germination of dicotyledonous seeds. In many species the covering layers impose a physical constraint to radicle protrusion, a phenomenon known as 'coat-enhanced' dormancy (Hilhorst, 1995; Bewley, 1997*a*). In cases where endosperm rupture is the germination-limiting process, weakening of the micropylar endosperm surrounding the radicle tip seems to be required for radicle protrusion and is likely to be achieved by cell-wall hydrolysis by the action of hydrolytic enzymes (Ni and Bradford, 1993; Black, 1996; Bewley, 1997*b*).

Considerable evidence suggests a causal role for class I β-1,3-glucanases (βGLU I) in the emergence of the radicle during tobacco seed germination (Leubner-Metzger and Meins, 1999, 2000). Rupture of the testa precedes rupture of the micropylar endosperm, which is the limiting step of tobacco seed germination (Arcila and Mohapatra, 1983). βGLU I is transcriptionally induced in the micropylar endosperm after testa rupture, but just prior to the onset

[3] To whom correspondence should be addressed at Freiburg. Fax: +49 761 203-2612. E-mail: leubner@uni-freiburg.de

Abbreviations: ABA, abscisic acid; *β*GLU I, class I *β*-1,3-glucanase; DAP, days after pollination; GA, gibberellins.

of endosperm rupture (Leubner-Metzger *et al.*, 1995). βGLU I induction and endosperm rupture are tightly linked in response to plant hormones and environmental factors known to affect the incidence and timing of germination (Leubner-Metzger *et al.*, 1996, 1998). For example, ABA delays endosperm rupture of seeds imbibed in the light and inhibits βGLU I accumulation in a concentration-dependent manner (Leubner-Metzger *et al.*, 1995). Finally, sense transformation of tobacco with a chimeric ABA-inducible βGLU I transgene caused overexpression of βGLU I in seeds and promoted endosperm rupture of mature seeds and of ABA-treated after-ripened seeds (Leubner-Metzger and Meins, 2000). Taken together, these results showed that βGLU I substantially contributes to endosperm rupture.

In the present study antisense transformation was used and indirect evidence was provided for a second, novel site of βGLU I action on the release of dormancy during after-ripening of seed.

Materials and methods

Plasmid construction and plant transformation

The chimeric antisense-βGlu I constructs KAG4, GAG2 and GAG3 (Fig. 1) are transcriptional fusions of three different promoters to the 1.3 kb cDNA containing the entire 1111 bp coding and 184 bp non-coding sequence of βGLU I in reverse orientation (Shinshi *et al.*, 1988; Beffa *et al.*, 1993). *Eco*RI-*Bam*HI DNA fragments containing the promoters were ligated to a 4.1 kb *Bam*HI-*Eco*RI-*Eco*RI fragment containing the cauliflower mosaic virus (CaMV) 35S terminator and the vector of the βGLU I-antisense plasmid described previously (Beffa *et al.*, 1993). The 2.7 kb *Eco*RI-*Bam*HI *Cat1* promoter (Suzuki *et al.*, 1994, 1995) yielded construct KAG4, and the 1.7 kb full-length *Eco*RI-*Eco*RI-*Bam*HI and 0.5 kb proximal *Eco*RI-*Bam*HI promoters of the *Glb* genomic clone (Hart *et al.*, 1993) yielded the constructs GAG2 and GAG3, respectively. The binary vector pCIB200 (Neuhaus *et al.*, 1992) was linearized in the polylinker with *Kpn*I and *Eco*RI and ligated to *Kpn*I-*Eco*RI fragments of the chimeric antisense-βGLU I genes. The resulting plasmids pKAG4, pGAG2 and pGAG3 carry the modified T-DNAs (Fig. 1) with the chimeric antisense-βGLU I genes transcribed in the same direction as the chimeric *Nos-NptII* gene. The methods for introducing the pCIB200 expression vectors into *Agrobacterium tumefaciens*, Ti-plasmid transformation of *Nicotiana tabacum* L. cv. Havana 425 leaf discs, regeneration of plants, and segregation tests using the kanamycin-resistance marker have been described (Beffa *et al.*, 1993). TCIB1 transformants obtained with the empty-vector plasmid pCIB200 were used as controls. Segregation tests were performed with S_1 seeds obtained by self-fertilization of independent primary transformants. Homozygous, monogenic S_2 seeds were obtained by self-fertilization of S_1 plants using kanamycin-resistance as the marker.

Analysis of germination, proteins and RNA

Seed from mature capsules of wild-type or transformed Havana 425 tobacco were used either at *c.* 40 DAP ('fresh' seed) or after at least 6 months of dry storage at *c.* 22 °C and *c.* 30% moisture content in the dark (after-ripened seed), as indicated. Germination analyses were performed as described earlier (Leubner-Metzger *et al.*, 1998). In brief, 100–150 seeds were sown in plastic Petri dishes containing filter paper wetted with a nutrient solution supplemented as indicated with 50 μg ml^{-1} kanamycin and 100 μg ml^{-1} Claforan. Petri dishes were incubated at 25 °C in continuous white light (3000 lx, Philips 'TL'D 35W/33 lamps) or in darkness. After scoring for germination, seeds were stored at −80 °C for subsequent analysis. Procedures for extracting proteins, assays for enzyme activity, immunoblot

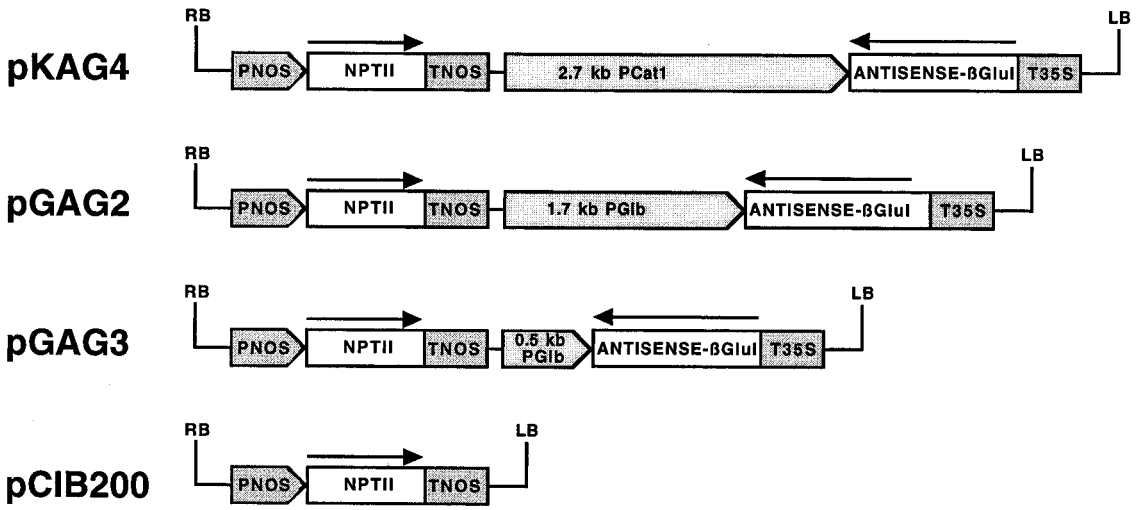

Fig. 1. Schematic representation of antisense class I β-1,3-glucanase (βGLU I) expression vectors used for tobacco transformation. Transcriptional fusions of the castor bean *Cat1* promoter (PCat1), the tobacco βGLU I B gene (*Glb*) full length promoter (1.7 kb PGlb), and the truncated proximal *Glb* promoter (0.5 kb PGlb) with a 1.3 kb βGLU I cDNA in reverse orientation and the CaMV 35S terminator (T35S) constitute the antisense βGLU I constructs of pKAG4, pGAG2 and pGAG3, respectively. The empty-vector pCIB200 was used to generate control transformants. The chimeric neomycin phosphotransferase gene (*NPT*II) confers kanamycin resistance and is under the control of the nopaline synthase promoter (PNOS) and terminator (TNOS). The right (RB) and left (LB) T-DNA borders are indicated.

analysis, protein determination, preparation of total RNA, and RNA-blot hybridization have been described previously (Leubner-Metzger and Meins, 2000).

Results

Testa rupture is delayed in antisense βGLU I transformant seeds

Tobacco plants were transformed with antisense constructs containing 1.3 kb of a tobacco βGLU I cDNA in reverse orientation regulated by the *Cat1* gene promoter (KAG4), a full-length 1.7 kb βGLU I gene promoter (GAG2), and a 0.5 kb minimal βGLU I gene promoter (GAG3) (Fig. 1). Reporter-gene experiments showed that the full-length and minimal βGLU I promoters confer high- and low-level endosperm-specific expression in germinating tobacco seeds, respectively (Leubner-Metzger *et al*., 1998). The *Cat1* promoter is known to confer endosperm-specific transgene expression in germinating tobacco seeds (Suzuki *et al*., 1995; Leubner-Metzger and Meins, 2000). In the initial screen, after-ripened seed from self-fertilized primary transformants were scored for percentage endosperm rupture at 65 h after the start of imbibition in control medium. Under these conditions all of the 27 TCIB1 control lines had germination percentages greater than 20%. In contrast, 28% of 66 TGAG2 lines, 7% of 58 TGAG3 lines, and 23% of 56 TKAG4 lines had germination percentages less than 20% at the 65 h time point.

Four independent lines of each antisense transformant that showed delayed germination were used in time-course studies. The results in Table 1 show that both testa and endosperm rupture was delayed by from 3–12.5 h in S_1 seed populations from different antisense lines. Detailed time-course studies were made with after-ripened seed of homozygous, monogenic lines derived from the transformants TGAG2-24 and TKAG4-31, which showed substantial delays (12.5 h and 8 h) in the S_1 generation screen (Table 1). Figure 2A and B shows that testa and endosperm rupture was delayed by *c*. 9 h in TGAG2-24 and *c*. 11 h in TKAG4-31 relative to TCIB1-2. Thus, delay in endosperm rupture of after-ripened antisense βGLU I seeds is strictly correlated with the delay in testa rupture. Although the possibility can not be excluded that βGLU I antisense transformation affects both events independently, the authors favour the hypothesis that the delay in endosperm rupture is the consequence of the delay in testa rupture.

Figure 2C shows that the onset of βGLU activity accumulation in the homozygous, monogenic antisense βGLU I seeds (lines TGAG2-24 and TKAG4-31) was delayed relative to TCIB1-2. This delay was comparable to the delay in testa rupture. Moreover, the βGLU activity detected in seed sampled at the time of 50% germination was comparable in control and antisense lines. It could be argued that antisense-mediated reduction in βGLU I is compensated for by the production of antigenically distinct 'ersatz' enzymes as reported for virus-infected leaves (Beffa *et al*., 1993). This was ruled out by antibody inhibition experiments and immunoblot analyses, which indicated that all the βGLU activity in the antisense-seeds could be accounted for by βGLU I (data not shown). A single 1.6 kb βGLU I transcript of the size expected for tobacco βGLU I mRNA (Mohnen *et al*., 1985) was detected by RNA-blot hybridization using a βGLU I cDNA probe. No signals were detected for the slightly smaller antisense transcript described in leaves of antisense βGLU I transformants (Neuhaus *et al*., 1992). Quantification of the hybridization signals revealed that the accumulation of βGLU I mRNA was comparable in seeds of the TCIB1-2 and TGAG2-24 transformants and somewhat less in the TKAG4-31 transformants (Fig. 2D). Measurements performed with leaf tissue samples from mature homozygous, monogenic antisense and empty-vector control plants similarly revealed no reduction of βGLU activity, βGLU I antigen and mRNA contents (data not shown). Thus, there was no consistent inhibition of βGLU I expression associated with leaves and germinating seeds during endosperm rupture in homozygous antisense lines. Nevertheless,

Table 1. *Delay of testa rupture and endosperm rupture of after-ripened S_1 seeds of independent antisense βGLU I transformants*

Line[a]	Number of transgene loci[b]	Time (h) of 50% rupture[c]		β-1,3-glucanase activity (pkat/seed)[d]
		Testa	Endosperm	
TCIB1-2	1	32.5	61.0	2.68
TCIB1-6	2	32.0	59.5	2.43
TCIB1-9	2	32.5	60.0	2.70
TCIB1-10	1	33.0	61.0	2.88
TGAG2-24	1	45.0 (12.5)	73.0 (12.5)	1.48
TGAG2-50	1	37.0 (4.5)	65.0 (4.5)	–[e]
TGAG2-62	2	36.0 (3.5)	66.5 (6.0)	1.30
TGAG2-66	1	38.0 (5.5)	66.5 (6.0)	2.80
TGAG3-38	≥3	37.0 (4.5)	68.5 (8.0)	–
TGAG3-45	≥3	36.0 (3.5)	65.5 (5.0)	–
TGAG3-47	1	35.5 (3.0)	64.5 (4.0)	2.10
TGAG3-58	1	36.0 (3.5)	64.5 (4.0)	–
TKAG4-30	2	38.0 (5.5)	68.5 (8.0)	–
TKAG4-31	1	38.5 (6.0)	68.5 (8.0)	1.56
TKAG4-48	1	39.0 (6.5)	68.5 (8.0)	2.60
TKAG4-50	1	41.0 (8.5)	69.0 (8.5)	2.30

[a]Independent primary transformants of Havana 425 tobacco carrying antisense βGLU I transgene loci (TGAG2, TGAG3, TKAG4) or empty-vector loci (TCIB1).

[b]As judged from the segregation of the K_m^R marker in S_1 seeds.

[c]Determined from the time-course of rupture for segregating S_1 populations of 100–150 after-ripened seeds imbibed in continuous light in control medium supplemented with antibiotics. Similar results were obtained when antibiotics were not added. The delay relative to the mean of TCIB1 lines is shown in parenthesis.

[d]Seeds sampled at the time of 50% endosperm rupture.

[e]Not determined.

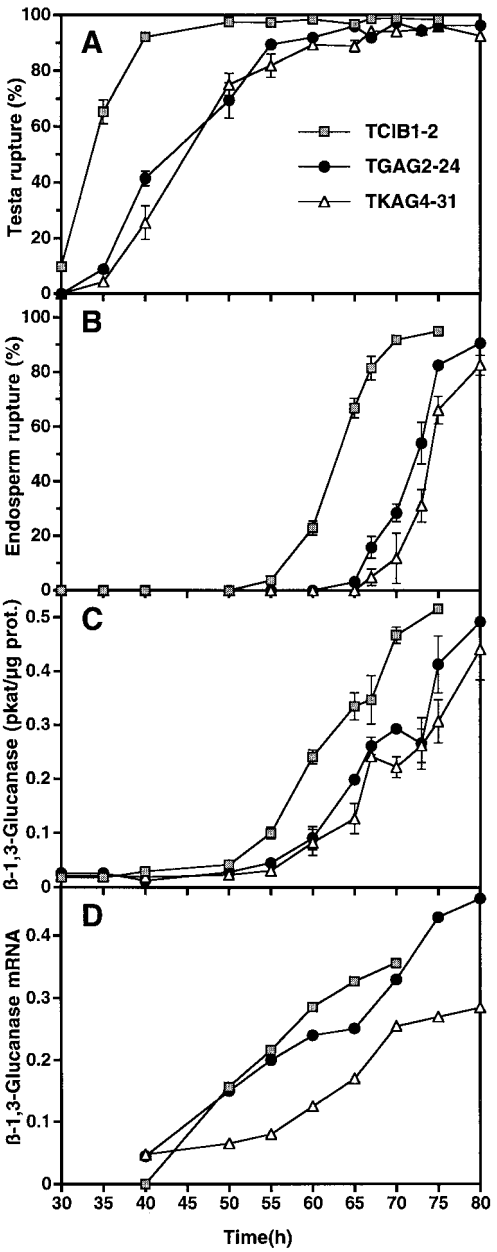

Fig. 2. The effect of antisense βGLU I transformation on the time-course of βGLU I induction during germination of after-ripened homozygous seed. (A) The incidence of testa rupture expressed as a percentage scored with time after the start of imbibition in continuous light in control medium. Homozygous, monogenic S_2 seed populations of independent βGLU I-antisense lines TGAG2-24 (β*GLU I* promoter) and TKAG4-31 (*Cat1* promoter) and of empty-vector line TCIB1-2 were used. (B) The incidence of endosperm rupture. (C) The accumulation of βGLU enzyme activities expressed in pkat μg^{-1} protein. (D) The accumulation of the 1.6 kb βGLU I mRNA expressed as arbitrary PhosphoImager units per seed. The signals detected and quantified by RNA-blot hybridization using additional seed samples from the experiment described in (A) are corrected for RNA loading based on the 18S ribosomal RNA signals. (A–C) Mean values ±SE of two samples each with 100–150 seeds are presented (30 h one sample); SE-values ≤2.0% and ≤0.01 pkat μg^{-1} protein are not drawn.

independent antisense transformants made with these promoters showed a substantially delayed germination of after-ripened seed and was correlated with promoter strength. The fact that this delay could be accounted for by the delay in testa rupture, which is prior to βGLU I induction, led to the investigation of antisense effects on the release of dormancy during after-ripening.

Effects of βGLU I antisense transformation on after-ripening and photodormancy

The germination percentages were measured in the light at a fixed time after the start of imbibition of fresh mature seeds harvested 40 d after pollination (DAP) and of after-ripened seeds stored at room temperature for at least 6 months after harvest. The final incidence of germination in the light of all the lines was >95%, implying that the germination percentages scored at a fixed time reflect the rate of germination. Table 2 shows that fresh sense seeds (TKSG7) germinated at approximately 1.5-fold higher percentages (Leubner-Metzger and Meins, 2000), whereas fresh antisense seeds germinated at the same percentages as control seeds. In contrast, after-ripened antisense seeds germinated at *c.* 9-fold lower percentages compared to control and sense seeds, which germinated at comparable percentages. Thus, in light-imbibed seeds overexpression of βGLU I replaced the promoting effect of after-ripening on germination; whereas after-ripening appears to be required for the inhibitory effects of antisense βGLU I transformation on seed germination.

After-ripening contributes to the release of photodormancy and this effect varies greatly for different seed batches as reported for several tobacco cultivars (Kasperbauer, 1968; Leubner-Metzger and Meins, 2000). Freshly harvested Havana 425 tobacco seeds do not germinate in the dark, and neither testa nor endosperm rupture occur even after prolonged periods of time (Leubner-Metzger *et al.*, 1996). Sense βGLU I transformation did not have detectable effects on either photodormancy of fresh seed or on the after-ripening-mediated release of photodormancy (Leubner-Metzger and Meins, 2000). Table 2 shows that antisense βGLU I transformation effectively blocked the release of photodormancy during after-ripening. While these germination percentages represent the final incidence of germination during dark-incubation, complete germination can be achieved by subsequent transfer of the dark-incubated seeds to the light.

Discussion

Sense and antisense transformation of tobacco revealed at least two sites of βGLU I action on seed germination and dormancy. The tobacco embryo is surrounded by three to five layers of thick-walled endosperm cells and an outer

Table 2. *Effect of after-ripening on endosperm rupture and photodormancy of sense and antisense βGLU I seeds*

Lines[a]	% Endosperm rupture in continuous light[b]		% Non-photodormancy[c]	
	'Fresh' seed	After-ripened seed	'Fresh' seed	After-ripened seed
Wild type	49.8 ± 5.2	79.2 ± 6.8	0.4 ± 0.2	50.3 ± 16.5 (0.0–80.6)
TCIB1-2	49.7 ± 2.1	81.4 ± 5.2	0.3 ± 0.2	40.0 ± 12.6 (9.1–80.1)
TKSG7-32	74.4 ± 2.1	79.0 ± 2.4	0.3 ± 0.3	29.7 ± 13.5 (3.0–63.6)
TKSG7-38	77.1 ± 2.1	73.5 ± 0.1	0.9 ± 0.2	39.0 ± 18.9 (0.0–82.4)
TKSG7-43	75.3 ± 3.6	75.8 ± 7.4	0.4 ± 0.2	43.6 ± 19.6 (9.4–90.2)
TGAG2-24	48.8 ± 5.4	15.8 ± 4.5	0.2 ± 0.1	7.8 ± 2.6 (3.0–15.5)
TGAG2-19	–[d]	9.4 ± 0.7	–	1.8 ± 0.7 (0.0–2.9)
TKAG4-31	47.3 ± 6.2	4.9 ± 1.7	0.6 ± 0.3	3.2 ± 2.3 (0.0–10.2)
TKAG4-50	–	11.8 ± 2.1	–	5.6 ± 1.5 (1.8–9.6)

[a]Wild-type and independent, homozygous, monogenic vector-control (TCIB1), sense βGLU I (TKSG7) and antisense βGLU I (TGAG2, TKAG4) tobacco lines selected from the S_2 seed generation. (Data for sense seeds obtained in the same experiment are from Leubner-Metzger and Meins, 2000.)

[b]Mean ± SE of 'fresh' (directly after harvest) and after-ripened (≥ 6 months of dry storage) seed from 3–6 capsules scored 72 h and 67 h after the start of imbibition in continuous light in control medium without antibiotics, respectively.

[c]Mean ± SE of 'fresh' and after-ripened seed from 3–6 capsules scored after 10 d incubation in the dark in control medium without antibiotics. The range obtained with different capsules is shown in parenthesis.

[d]Not determined.

testa of maternal origin consisting of cutinized and lignified dead cells and a living inner parenchyma layer (Avery, 1933; Matzke *et al*., 1993). In members of the Solanaceae (e.g. tomato, tobacco, pepper, and *Datura* ssp.) the endosperm is a mechanical restraint to radicle protrusion, and germination seems to depend on both endosperm weakening by hydrolytic enzymes and sufficient growth of the embryo to overcome this constraint (Ni and Bradford, 1993; Bewley, 1997*b*). Ultrastructural studies suggest that the endospermic hole formed at the micropylar end of germinating tobacco seeds results from endosperm 'dissolution' rather than from the 'pushing' action of radicle growth (Arcila and Mohapatra, 1983). Earlier (Leubner-Metzger and Meins, 2000) sense transformation was used with a chimeric βGLU I gene regulated by the ABA-inducible *Cat1* promoter which caused overexpression of βGLU I in imbibed tobacco seeds and promoted endosperm rupture of mature seeds and of ABA-treated after-ripened seeds. While these results provided direct evidence that βGLU I has a role in endosperm rupture, they also showed that sense transformation does not affect the timing of testa rupture during imbibition in the light and the release of photodormancy during after-ripening.

The failure to find antisense-mediated reduction of βGLU I expression during endosperm rupture and in leaves is puzzling. The βGLU I cDNA in reverse orientation that was used, when regulated by the CaMV 35S RNA promoter, effectively reduced βGLU I expression in tobacco leaves (Beffa *et al*., 1993). Insufficient promoter strength of the *βGLU I* and *Cat1* promoter compared to the CaMV 35S RNA promoter could be a reason for the failure to find antisense-mediated reduction of βGLU I in leaves (Vögeli-Lange *et al*., 1994*b*; Suzuki *et al*., 1995). However, in germinating seeds, the promoters used directed expression of reporter genes in the endosperm (Suzuki *et al*., 1995; Leubner-Metzger *et al*., 1998) and the *Cat1* promoter caused an overexpression of βGLU I (Leubner-Metzger and Meins, 2000). 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. The important point is that numerous independent antisense transformants made with these promoters affected the release of photodormancy during after-ripening and showed a substantial incidence of delayed testa rupture of after-ripened seed during imbibition in the light, which was correlated with promoter strength. These results suggest a novel role of βGLU I action during after-ripening and photodormancy release.

The primary effect of antisense βGLU I transformation in after-ripened seeds was prior to the onset of testa rupture. A most intriguing observation was that antisense transformation did not affect germination of fresh tobacco seeds sampled *c*. 40 DAP, which is after maturation and the establishment of primary dormancy is complete (Yamaguchi-Shinozaki *et al*., 1990; Jakobsen *et al*., 1994; Jiang *et al*., 1996). βGLU I expression is not detectable during the early development or maturation of seeds (data not shown), or during early stages of imbibition (Leubner-Metzger *et al*., 1995). Antisense βGLU I transformation has no effect on germination in the light and on photodormancy of fresh seeds and, therefore, interferes with secondary seed dormancy rather than primary dormancy. Thus, it is plausible to assume that antisense transformation affects βGLU I expression sometime during after-ripening. This conclusion and the finding that overexpression of βGLU I in sense transformants replaced the effect of after-ripening on germination (Leubner-Metzger and Meins, 2000), leads to the proposal that after-ripening is mediated, at least in part, by

expression of βGLU I during prolonged dry storage of seed. βGLU I could act by direct cell wall degradation resulting in weakening or death of entire cell layers or indirectly by releasing elicitor-active β-1,3-glucan oligosaccharides that serve as signalling molecules in analogy to the situation during plant–pathogen interaction (Boller, 1995). This could directly modify the growth potential of the embryo or alter the characteristics of the testa tissue, which are known to affect seed dormancy in *Arabidopsis thaliana* (Debeaujon and Koornneef, 2000; Debeaujon *et al*., 2000). Knowledge about transcriptional and translational processes leading to gene expression during after-ripening is extremely limited. Although the metabolic activity of seeds in the dry state is very low, Comai and Harada have demonstrated that transcripts of some genes are elongated in nuclei isolated from dry seeds (Comai and Harada, 1990), suggesting that these genes are at least transcriptionally competent in the desiccated state. *In situ* mRNA hybridization of the *Arabidopsis* GEA1 gene revealed a tissue-specific expression pattern in dry seeds (Gaubier *et al*., 1993). The hypothesis that βGLU I is expressed and susceptible to antisense inhibition in dry seeds is supported by preliminary results showing that βGLU I protein and activity is sometimes detected in wild-type but not in antisense seeds sampled at different times during after-ripening (data not shown).

Little is known about the molecular basis for dormancy or the modulation of dormancy during after-ripening (Li and Foley, 1997; Bewley, 1997*a*). In many species, including tobacco, endogenous production of ABA is needed for establishing dormancy and for maintaining dormancy as well (Koornneef and Karssen, 1994; Rock and Quatrano, 1995; Li and Foley, 1997; Bewley, 1997*a*; Grappin *et al*., 2000). The endogenous ABA content of dry after-ripened seeds of *Nicotiana plumbaginifolia* is considerable lower compared to dry mature dormant seeds (Grappin *et al*., 2000). During after-ripening, decreasing ABA levels and possibly decreasing sensitivity to ABA eventually permits βGLU I expression needed for endosperm rupture during seed imbibition (Leubner-Metzger and Meins, 2000), but could also permit βGLU I expression in the dry state, which results in the release of dormancy. Decline in ABA content, decreased sensitivity to ABA and increased sensitivity to gibberellins (GA) is correlated with after-ripening of many species (Hilhorst, 1995; Li and Foley, 1997; Bewley, 1997*a*). While βGLU I expression in seeds is inhibited by ABA, it is promoted by light, GA, and ethylene (Leubner-Metzger *et al*., 1998; Leubner-Metzger and Meins, 1999). It was found that after-ripening contributes to the release of photodormancy of Havana 425 tobacco seeds as has been reported for other tobacco cultivars (Kasperbauer, 1968). Whereas sense transformation did not have detectable effects on photodormancy (Leubner-Metzger and Meins, 2000), antisense transformation greatly inhibited the release of photodormancy due to after-ripening. This raises the possibility that a contribution of βGLU I is necessary for the after-ripening-mediated release of photodormancy. However, βGLU I alone is not sufficient and other factors are needed 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 (Li and Foley, 1997; Bewley, 1997*a*). The fact that βGLU I expression is regulated by hormones and environmental factors (reviewed in Leubner-Metzger and Meins, 1999) suggests that βGLU I could be a key factor in modulating dormancy and germination in response to the environment.

Acknowledgements

We thank Masaharu Suzuki and Waltraud Schmidt-Puchta for kindly providing plasmids; Rosa Waldvogel and Monique Thomas for expert technical assistance; Sjoerd van Eeden and Markus Briker for care of plants; and Thomas Boller, Ramamurthy Baskar and Alejandro Iglesias for their critical comments.

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