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Hormonal Regulation of Dormancy in Developing Sorghum Seeds[1]

Haydée S. Steinbach*, Roberto L. Benech-Arnold, and Rodolfo A. Sánchez

Departamento de Producción Vegetal (H.S.S., R.L.B.-A.), and Departamento de Ecología (R.A.S.), IFEVA, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, 1417 Buenos Aires, Argentina

The role of abscisic acid (ABA) and gibberellic acid (GA) in determining the dormancy level of developing sorghum (*Sorghum bicolor* [L.] Moench.) seeds from varieties presenting contrasting preharvest sprouting behavior (Redland B2, susceptible; IS 9530, resistant) was investigated. Panicles from both varieties were sprayed soon after pollination with fluridone or paclobutrazol to inhibit ABA and GA synthesis, respectively. Fluridone application to the panicles increased germinability of Redland B2 immature caryopses, whereas early treatment with paclobutrazol completely inhibited germination of this variety during most of the developmental period. Incubating caryopses in the presence of 100 μM GA_{4+7} overcame the inhibitory effect of paclobutrazol, but also stimulated germination of seeds from other treatments. IS 9530 caryopses presented germination indices close to zero until physiological maturity (44 d after pollination) in control and paclobutrazol-treated particles. However, fluridone-treated caryopses were released from dormancy earlier than control and paclobutrazol-treated caryopses. Incubation in the presence of GA_{4+7} stimulated germination of caryopses from all treatments. Our results support the proposition that a low dormancy level (which is related to a high preharvest sprouting susceptibility) is determined not only by a low embryonic sensitivity to ABA, but also by a high GA content or sensitivity.

Preharvest sprouting is one of the main problems encountered in sorghum (*Sorghum bicolor* [L.] Moench.) production, particularly when seed maturation takes place under damp conditions. Although it is known that genetic variability for sprouting resistance exists, the underlying physiological mechanisms are poorly understood. Sprouting resistance in sorghum is related to the maintenance of a sufficient dormancy level during development (Steinbach et al., 1995). Indeed, although isolated embryos from both susceptible and resistant varieties can germinate equally well in water from early stages of development (i.e. 15 DAP or earlier), the germination capacity of intact developing caryopses from resistant varieties is much less than that observed for susceptible genotypes. This coat-imposed dormancy is the barrier preventing untimely germination.

The acquisition of dormancy has been shown in several species to be related to ABA action during seed development. In most seeds the ABA level increases steadily during development, reaching high values before ripening and then declining sharply (King, 1982). Tomato (Groot et al., 1991), Arabidopsis (Karssen et al., 1983), and maize (Neill et al., 1987) mutants deficient in ABA synthesis show substantially reduced peak ABA contents and almost no dormancy. Accordingly, the inhibition of ABA synthesis during early development of maize seeds blocks their entrance into dormancy (Fong et al., 1983), and the inclusion of fluridone in the incubation medium stimulates the germination of dormant sunflower embryos (Le Page-Degivry and Garello, 1992). On the other hand, the addition of physiological concentrations of ABA prevents precocious germination, blocks the expression of germination-specific enzymes, and promotes embryonic development (King, 1982; Triplett and Quatrano, 1982; Black, 1983, 1991; Quatrano et al., 1983). In previous work we found that ABA content during sorghum seed development did not differ between sprouting-resistant and -susceptible varieties (Steinbach et al., 1995); however, embryos from a susceptible variety were found to be 10-fold less sensitive to the inhibitory action of ABA than their resistant counterparts. Similar results were found in wheat, in which the differences in germination between susceptible and resistant varieties were related in part to differences in embryo sensitivity to ABA (Walker-Simmons, 1987). Although our results suggest that ABA participates in the control of dormancy during sorghum seed development, ABA action does not fully explain the different behavior of immature caryopses from varieties with contrasting susceptibility to preharvest sprouting (Steinbach et al., 1995).

The germination-promoting effect of GAs is well documented for mature seeds from a number of species (Lona, 1956; Karssen et al., 1989; Hilhorst, 1995; Karssen, 1995). Although GAs are known to accumulate in developing seeds from many species (for review, see Khan, 1982; Sponsel, 1983; García-Martínez et al., 1987) and some studies have reported that exogenously applied GAs can induce precocious germination of immature embryos (Norstog and Klein, 1972), their role in determining the germination capacity of developing seeds is by far less clear than in the case of ABA. Several authors have proposed that the dormancy level of a seed population depends on the balance between the action of promotive (GAs, kinetins) and inhibitory (ABA) hormones (Luckwill, 1952; Khan, 1982). On the other hand, Karssen and Lacka (1986) concluded that the

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* Corresponding author; e-mail rvmirall@criba.edu.ar; fax 54–1–521–1384.

Abbreviation: DAP, days after pollination.

GAs do not play any significant role in the establishment of or exit from dormancy based on results with GA-deficient mutants. The same authors proposed that, in contrast to the hormone balance theory, GAs and ABA do not interact directly—that GAs are required for germination once dormancy is lost, whereas ABA levels influence the depth of dormancy during development. Although the work with mutants is valuable, it has so far been carried out with only a few species, which precludes wide generalizations (Bewley and Black, 1994).

In this paper we assess the impact on dormancy level of modifying ABA and GAs syntheses during development of immature sorghum caryopses from varieties with contrasting preharvest sprouting behavior.

MATERIALS AND METHODS

Two different varieties of sorghum (*Sorghum bicolor* [L.] Moench.) presenting contrasting behavior to preharvest sprouting were used in this study: Redland B2 (susceptible) and IS 9530 (resistant). Both varieties were sown in the experimental field of the Facultad de Agronomía (Universidad de Buenos Aires, Argentina) (34°25′ S; 58°25′ W) on December 19, 1994, in three randomized blocks. Plant density was around 8 to 12 plants m^{-2}. Control of weeds, insects, and diseases was carried out following the schedule used under production conditions. The crop was irrigated when necessary to avoid water stress. Each plant was labeled with its flowering date when pollen had been released in florets from the upper two-thirds of the panicle. To minimize variation, only spikelets from the middle one-third of the panicle were used for the determinations.

Treatments

Twelve panicles from each block and from each variety were sprayed with fluridone (Sonar, Elanco Products, Indianapolis, IN) at a concentration of 200 μL of 42% fluridone dissolved in 500 mL of water on 3, 5, 8, 11, and 14 DAP. An additional 12 panicles from each block and from each variety were sprayed with paclobutrazol (ICI, Buenos Aires, Argentina) at a concentration of 1 g of paclobutrazol in 1 L of water on 3 and 7 DAP. Beginning on 16 DAP, two or three plants from each variety, treatment, and block were harvested on each sampling date. Fifty caryopses were weighed, dried at 90°C for 72 h, and weighed again to determine dry weight and moisture content.

Germination Tests

Control, fluridone-treated, and paclobutrazol-treated panicles from both varieties were harvested on 16, 23, 28, 36, 44, and 54 DAP; and 50 caryopses were incubated in Petri dishes containing 6 mL of either distilled water or 100 μM GA_{4+7} at 25°C. Germination embryo growth was counted daily for 12 d and a germination index (*GI*) was constructed as follows:

$$GI = (n1 \times 12 + n2 \times 11 + \ldots + n12 \times 1)/50,$$

where $n1$ through $n12$ are the number of caryopses germinated on d 1 and subsequent days (Walker-Simmons, 1987).

Embryonic Sensitivity to ABA

On 36 DAP, embryos from control, fluridone-treated, and paclobutrazol-treated caryopses from both varieties were incubated in a range of ABA concentrations (0, 0.5, 5, and 50 μM) at 25°C. Germination (embryo growth) was counted daily for 12 d and the same germination index described above was constructed.

ABA Quantification in Embryos

ABA content was determined in embryos collected at different stages of development with a radioimmunoassay that uses the monoclonal antibody AFRC MAC 252 (Quarrie et al., 1988), as described previously (Steinbach et al., 1995).

RESULTS

Dry Matter Accumulation and Moisture Content in Developing Seeds

No alterations were found in dry matter accumulation or in moisture content as a result of the inhibitor treatments in developing seeds from either variety (data not shown). Maximum dry weight was attained on 36 DAP in Redland B2 and on 44 DAP in IS 9530.

Germination of Developing Seeds

Fluridone application during early stages of development (3–14 DAP) increased the germination capacity of Redland B2 immature caryopses from 28 DAP on in relation to that observed for control caryopses (Fig. 1A). Conversely, caryopses from panicles that had been treated with paclobutrazol presented germination indices close to zero during most of the developmental period (Fig. 1A). This inhibitory effect of an early paclobutrazol application was overcome by incubation of the harvested caryopses in the presence of 100 μM GA_{4+7}, suggesting that paclobutrazol imposed a deeper dormancy level on immature caryopses through inhibition of GA synthesis (Fig. 1B). Moreover, the germination of caryopses from control and fluridone-treated panicles was also stimulated by exogenous GA (Fig. 1B). Redland B2 and IS 9530 control caryopses were incubated in the presence of 100 μg mL^{-1} paclobutrazol at different stages of development. No differences in seed germination were observed in relation to that from control caryopses incubated in distilled water (Table I). Therefore, it is unlikely that the germination of caryopses from paclobutrazol-treated panicles was a result of the inhibitor remaining in the developing seeds.

Germination indices for IS 9530 caryopses were very low during most of the developmental period regardless of the treatment applied to the panicle after pollination (Fig. 1C). However, fluridone-treated caryopses became germin-

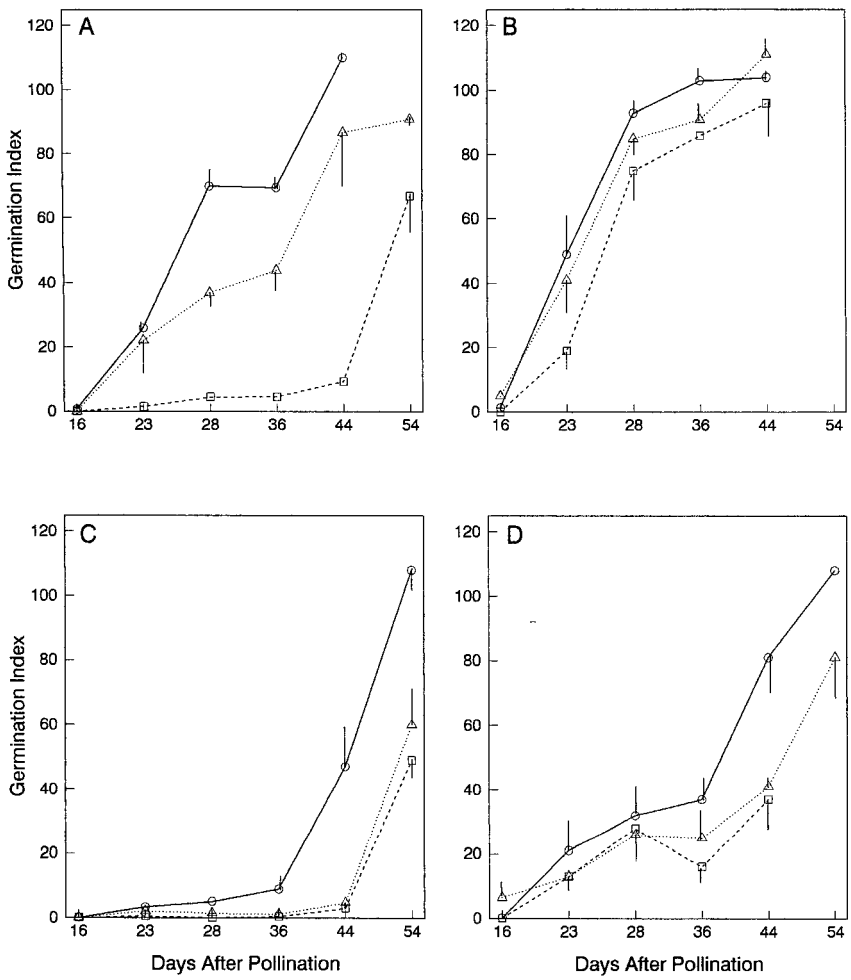

Figure 1. Germination indices of control (△), fluridone-treated (○), and paclobutrazol-treated (□) Redland B2 (A and B) and IS 9530 (C and D) caryopses harvested at different times after pollination and incubated in distilled water (A and C) or in 100 μM GA_{4+7} (B and D). Vertical bars are mean SE when larger than the symbol.

able earlier than did control and paclobutrazol-treated caryopses (Fig. 1C). Paclobutrazol did not modify caryopsis behavior in relation to that of control seeds of this variety (Fig. 1C). Incubation in the presence of 100 μM GA_{4+7} stimulated germination of caryopses from all treatments, but to a lesser extent than in the case of Redland B2 seeds (Fig. 1D).

To determine the extent to which differences in dormancy level between caryopses from both varieties were determined by a different balance of ABA and GA, the germination indices from fluridone-treated IS 9530 caryopses incubated in presence of GA_{4+7} were plotted together with indices corresponding to Redland B2 control caryopses. As is shown in Figure 2, the IS 9530 curve matched almost exactly the Redland B2 curve throughout the entire developmental period. This resemblance shows that the behavior of Redland B2 caryopses can be reproduced in IS 9530 by reducing the IS 9530 endogenous ABA content or by supplying IS 9530 caryopses with GAs. Conversely, the germination indices of Redland B2 caryopses

Table I. *Germination percentage after 48 h of incubation obtained from control Redland B2 and IS 9530 caryopses harvested at different stages of development and incubated in distilled water or in 100 μg mL^{-1} paclobutrazol*

NS, No significant difference ($P < 0.05$) between treatments within the same stage of development.

DAP	Redland B2			IS 9530		
	Control	Paclobutrazol		Control	Paclobutrazol	
	Germination					
16	0	0	NS	0	0	NS
23	0	0	NS	0	0	NS
28	1.8	4.5	NS	0	0	NS
36	26.4	21.3	NS	1.2	0.6	NS
44	63.6	71.4	NS	0.6	1.3	NS

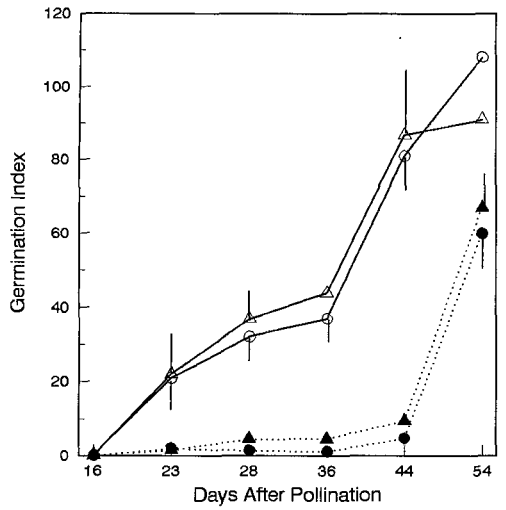

Figure 2. Germination indices of Redland B2 control (△), Redland B2 paclobutrazol-treated (▲), and IS 9530 control (●) caryopses incubated in distilled water, and IS 9530 fluridone-treated caryopses incubated in 100 μM GA_{4+7} (○) plotted against time after pollination. Vertical bars are mean SE when larger than the symbol.

from paclobutrazol-treated panicles were very similar to those from IS 9530 controls (Fig. 2).

Embryonic Sensitivity to ABA

To investigate the extent to which changes in germination caused by the application of fluridone and paclobutrazol were through changes in embryonic sensitivity to ABA, 36-DAP embryos were incubated in the presence of increasing ABA concentrations. In the case of Redland B2, embryos from fluridone-treated panicles presented less sensitivity to ABA than did controls (Fig. 3A). Indeed, it was necessary to increase the ABA concentration to 50 μM to inhibit the germination of embryos from fluridone-treated panicles to the same extent as that which occurred with 5 μM ABA in control embryos (Fig. 3A). Embryos from paclobutrazol-treated caryopses presented an intermediate pattern of response with a sensitivity that was not different from that presented either by fluridone-treated or control embryos (Fig. 3A). In the case of IS 9530 embryos, significant differences in sensitivity were found only between embryos from fluridone-treated and paclobutrazol-treated caryopses incubated at an ABA concentration of 5 μM (Fig. 3B).

ABA Levels in Embryos during Development

ABA levels in embryos of both varieties were sensitively affected by the application of fluridone. In Redland B2 embryos, fluridone treatment reduced ABA content to one-third that of the control on 23 and 36 DAP (Fig. 4A). In contrast, ABA content measured in paclobutrazol-treated embryos was not different from that detected in control embryos. ABA content of IS 9530 embryos was affected by fluridone application to a larger extent than that of Redland B2 embryos. From 23 DAP on, ABA content in fluridone-treated embryos was one-third to one-half that measured in control embryos (Fig. 4B).

DISCUSSION

In the two sorghum varieties used in this study, dormancy was imposed at an early ontogenic stage (before 16 DAP) and was gradually lost throughout development. In agreement with previous work with this material (Steinbach et al., 1995), Redland B2 caryopses were released from dormancy earlier than those of IS 9530. The difference in time to exit from dormancy explains their contrasting resistance to preharvest sprouting. Early applications (up to 14 DAP) of ABA and GA inhibitors to the panicles markedly affected the temporal pattern of dormancy release in both varieties. Thus, inhibiting ABA synthesis with fluridone advanced the exit from dormancy, whereas dormancy was extended when GA synthesis was inhibited with paclobutrazol (Fig. 1). The dormancy level of immature caryopses appears to have resulted from the balance between ABA and GA actions. This was particularly noticeable in Redland B2, in which early applications of either fluridone or paclobutrazol modified dormancy in opposite directions. The effect of inhibiting ABA synthesis in sorghum is consistent with current knowledge about the role of ABA in the onset of dormancy; ABA-deficient mutants

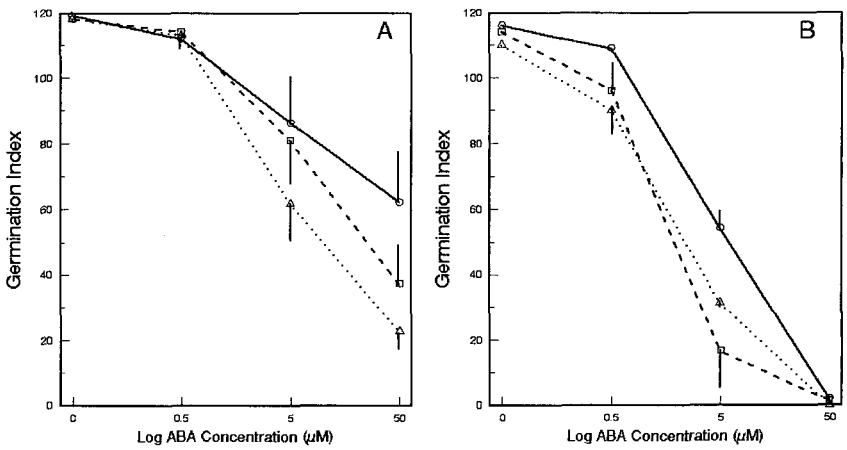

Figure 3. Germination indices of 36-DAP Redland (A) or IS 9530 (B) embryos isolated from control (△), fluridone-treated (○), and paclobutrazol-treated (□) caryopses and incubated under different ABA concentrations. Vertical bars are mean SE when larger than the symbol.

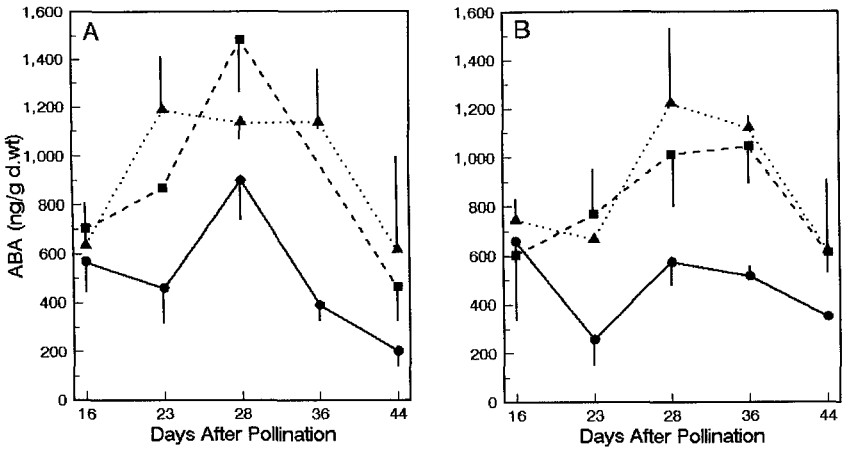

Figure 4. ABA content (ng/g dry weight) as a function of time after pollination in embryos from control (▲), fluridone-treated (●), and paclobutrazol-treated (■) Redland B2 (A) and IS 9530 (B) caryopses. Vertical bars are mean SE when larger than the symbol. d.wt, Dry weight.

of tomato and *Arabidopsis thaliana* both have very low levels of dormancy (Karssen et al., 1983; Groot and Karssen, 1992). Lowering ABA levels with fluridone has been shown to reduce dormancy in maize (Fong et al., 1983) and sunflower (Le Page-Degivry et al., 1992) seeds. In addition, changes in dormancy produced by fluridone could, to a certain extent, be related to its effects on embryonic sensitivity to ABA (Fig. 3). Le Page-Degivry and Garello (1992) also showed that fluridone reduces the sensitivity of sunflower embryos to ABA. We do not know the point up to which the decrease in embryonic sensitivity to ABA was a direct effect of fluridone or a consequence of the lowering of ABA content. It is known that endogenous hormone levels often interfere with tests of sensitivity to applied growth regulators (Karssen and Lacka, 1986). However, the application of fluridone reduced ABA levels in about the same proportion in both varieties of sorghum, whereas the effect on embryonic sensitivity to ABA was larger in Redland B2. This suggests that there may be a direct effect on sensitivity.

The literature is less clear with respect to the importance of GAs on the entrance and exit from dormancy during seed development. It is known that in developing seeds there is an intense synthesis of GAs (Khan, 1982) and that precocious germination of barley embryos can be induced by exogenous GA_3 (Norstog and Klein, 1972). Our results show that sorghum embryos are responsive to exogenous GAs from an early stage of development (Fig. 1). On the other hand, work with mutants deficient in GAs has led to the proposition that both the depth of dormancy and its reduction through after-ripening are independent of the GA level (Karssen and Lacka, 1986). According to this proposition, GAs are necessary for germination once dormancy has been removed. So far, the low dormancy level in immature seeds associated with preharvest sprouting susceptibility has been discussed only in terms of ABA action (Walker-Simmons, 1987; Steinbach et al., 1995). Our results, however, indicate that a role for GAs should be considered.

A very early treatment of the developing panicles (3 DAP) with paclobutrazol had a most significant impact on the behavior of immature caryopses up to 36 DAP. The caryopses of the susceptible variety harvested from panicles treated with paclobutrazol behaved similarly to those of the resistant variety developing in control plants (Fig. 2). It could be asked whether paclobutrazol action took place mainly during the early stages of development or whether the effects were due to the inhibitor that persisted in the seed tissues and acted during the germination test. However, the inclusion of paclobutrazol concentrations in the incubation medium of caryopses harvested at different stages of development did not have any effect on germination (Table I). These results suggest that any action of paclobutrazol must have taken place at earlier stages of development rather than during germination tests of harvested seeds. Although we have not measured the impact of paclobutrazol on GA levels in sorghum caryopses, it can be very effective even when applied early during seed development (García Martinez et al., 1987).

Taken together, the present results and those of previous papers (Benech-Arnold et al., 1995; Steinbach et al., 1995) support the following working hypothesis: the higher level of dormancy of IS 9530 caryopses results from (a) a higher embryonic sensitivity to ABA, and (b) lower GA content and sensitivity during development. This antagonism of ABA and GAs in deciding seed behavior is reflected by the fact that the pattern of exit from dormancy of the resistant IS 9530 caryopses can be similar to that of the susceptible Redland B2 when their panicles are treated with fluridone and the seeds are supplemented with GA_{4+7} (Fig. 2).

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