Induction of Dormancy in Nondormant Seeds

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Abstract. A gibberellic acid (GA) biosynthesis inhibitor, tetacyclacis, induced dormancy in nondormant seeds of lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum annuum* L.), carrot [*Daucus carota* var. *sativus* (Hoffm.)], onion (*Allium cepa* L.), celery (*Apium graveolens* L.), and impatiens (*Impatiens novette*), as most of the seeds failed to germinate after washing under conditions that permitted germination before dormancy induction. In lettuce seeds, tetacyclacis and paclobutrazol were more effective in inhibiting germination in light than in darkness. A 16- to 24-h soak treatment with tetacyclacis was sufficient to induce dormancy in nearly all seeds. Tetacyclacis failed to induce dormancy if applied after 6 h presoak in water. Dormancy induced by tetacyclacis was released by GA₄₊₇ (a mixture of gibberellin A₄ and A₇), light, and moist-chilling treatments. When GA₄₊₇ was applied with tetacyclacis, dormancy induction was prevented under both favorable, e.g., 25C, and unfavorable, e.g., 5C, or low water potential (ψ), germination conditions. Unlike tetacyclacis, abscisic acid (ABA) failed to induce dormancy in lettuce seeds. Thermodormancy induction in lettuce seeds at 35C was prevented by fluridone. However, neither ABA nor tetacyclacis countered its effect. Dormancy was also induced in lettuce seeds by ancymidol, flurprimidol, or paclobutrazol. Dormancy induced by tetacyclacis in pepper, tomato, carrot, and onion seeds was released by GA₄₊₇ but not by irradiation or moist-chilling. Chemical names used: 5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo[5.4.10^{2,6,0}8,11]-dodeca-3,9-diene (tetacyclacis); 1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-3-pentanol (paclobutrazol); α -cyclopropyl- α -(4-methoxyphenyl)-5-pyrimidine methanol (ancymidol); α -(1-methyl)- α -[4-(trifluoromethoxy) phenyl]-5-pyrimidine-methanol (flurprimidol); 1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4 (1H)-pyridinone (fluridone).

Growth-retarding compounds such as (2-chloroethyl) trimethylammonium chloride (CCC), 1,1-dimethylpiperidinium chloride (DPC), 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine carboxylate (AMO-1618), and tributyl-2,4-dichloro-benzylphosphonium chloride (Phosphon D) have been used to reduce stem growth and lodging (Cathey, 1964; Jung and Rademacher, 1983). The mode of action of these growth regulators appears to be related to the inhibition of GA synthesis (before *ent*-kaurene), as simultaneous application of GA will cancel the growth-retarding effect (Rademacher, 1991). Highly active growth retardants such as ancymidol, tetacyclacis, paclobutrazol, and uniconazol have recently been developed (Izumi et al., 1984; Jung et al. 1980; Lever et al., 1982; Shive and Sisler, 1976) with high specificity for oxidative steps leading from *ent*-kaurene to *ent*-kaurenoic acid in the GA biosynthesis pathway (Coolbaugh et al., 1978; Jung and Rademacher, 1983).

Evidence has been presented for the primary role of GA in dormancy control and seed germination (Khan, 1971). Inhibitors of GA biosynthesis, particularly those inhibiting *ent*-kaurene oxidation, readily inhibit seed germination and the effect is reversed by GA. Gardner (1983) found that ancymidol inhibited the red-light-induced germination of 'Grand Rapids' lettuce seed, which was reversed by GA₃, and he suggested that the photoreceptor, phytochrome, might exert its effect on germination via control of GA level. Similarly, Karssen et al. (1989) showed that the light-promoted germination of *Arabidopsis thaliana* seeds was inhibited by 10 μ M tetacyclacis and the effect was reversed by 10 μ M GA₄₊₇. In another study by Nambara et al. (1991), dark germination

of mutant seeds of *Arabidopsis thaliana* was completely inhibited by 10 μ M uniconazol and the inhibition was reversed by GA₃.

Abscisic acid is often implicated in dormancy induction and maintenance (Karssen et al., 1989; Le Page-Degivry and Garello, 1992). In many studies, dormancy release is synonymous with germination (radicle protrusion), as is dormancy induction with germination inhibition. In this study, data show that GA biosynthesis inhibitors, unlike ABA, are capable of inducing dormancy in nondormant seeds of several crops, and that the induced dormancy can be released by GA₄₊₇ and other factors that mimic GA effect, under conditions both favorable and unfavorable for germination. These data reveal that induction, prevention, or release of dormancy can be modulated in the absence of germination.

Materials And Methods

The following seeds were obtained for this study: lettuce (*Lactuca sativa* L. cv. Emperor, Garnet, Empress, and Grand Rapids), onion (*Allium cepa* L. cv. Texas Early Grano), carrot (*Daucus carota* var. *sativus* cv. Long Emperor), celery (*Apium graveolens* L. cv. FM 1218), and tomato (*Lycopersicon esculentum* Mill. cv. Jackpot) from Ferry-Morse Seed Company, Modesto, Calif.; lettuce (cv. Mesa 659, Montello, and Ithaca) from Harris Moran Seed Company, Rochester, N.Y.; pepper (*Capsicum annuum* L. cv. California Wonder) from Petoseed Company, Saticoy, Calif.; and impatiens (*Impatiens novette*) from Zaadunie Research, Enkhuisen, Holland. All seeds were stored at 7C and 28% relative humidity (RH).

The GA biosynthesis inhibitors (tetacyclacis, paclobutrazol, flurprimidol, and ancymidol), GA₄₊₇, and fluridone were gifts. ($\pm$)-cis, trans- ABA (Sigma) contained equal amounts of + and - ABA and the concentrations reported are those for +ABA (biologically active). Stock solutions of GA synthesis inhibitors and fluridone were made by dissolving the chemicals in small acetone amounts [0.5% (v/v)], and water was added to obtain the needed volume. Final acetone concentration never exceeded 0.25% (v/v). At this

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concentration acetone had no inhibitory effect on germination or seedling development. Other chemicals were dissolved directly in water.

To determine if GA biosynthesis inhibitors and ABA induce dormancy, seeds in batches of 200 were soaked in darkness under various conditions and for different periods in 9-cm petri plates on two layers of filter paper (grade 1; Whatman LabSales, Hillsboro, Ore.) moistened with 7 ml of test solutions, prepared in either water or in -1.2 MPa (32.6 g/100 ml water) polyethylene glycol 8000 solution. Germination during this initial soak was counted. Nongerminated seeds were washed with excess water on a sintered glass funnel under suction. Seeds were either germinated (in three replicates of 50 seeds each) before or after drying (with forced air to original weight for 2 h in darkness at 25C) in continuous light (8 to 9 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) from cool white fluorescent lamps, or in darkness at 25C, in 5-cm petri plates lined with two layers of filter paper (grade 1; Whatman) moistened with 3 ml water or GA_{4+7} solution. Failure to germinate in darkness and/or light indicated that dormancy was induced.

To determine if moist-chilling would release the dormancy previously induced by GA biosynthesis inhibitors, dormant seeds (three replicates of 50 seeds each) were moistened with 3 ml water on two layers of filter paper and chilled at 5C in the dark. Following moist-chilling, seeds were germinated in darkness at 25C.

To determine the point during imbibition when dormancy is induced by tetcyclacis or ABA, lettuce seeds (in three replicates of 50 seeds each) were imbibed in water in darkness for up to 10 h before transfer to tetcyclacis or ABA solution for 24 h in darkness. These seeds were then washed and germinated in water as above.

Manipulations requiring darkness (adding test solution, washing, counting, drying, and transferring seeds) were conducted under a green safe light as described before (Samimy and Khan, 1983). The experiments reported here were repeated two or more times with similar results.

Results

Effect of GA biosynthesis inhibitors on germination in light and darkness. The GA biosynthesis inhibitor, tetcyclacis, strongly inhibited the germination of nondormant 'Mesa 659' lettuce seeds at 25C (Fig. 1A). A 50- μM concentration of tetcyclacis was needed for inhibition in the light, compared to 10 μM in darkness. Similarly, paclobutrazol at 10 to 100 μM was more effective in inhibiting germination in darkness than in light (Fig. 1B). Generally, if the lettuce seed germination remained inhibited for about 24 h in darkness by an inhibitor at a particular concentration, the seeds were rendered dormant. Differences in responsiveness to tetcyclacis in light and darkness were found also in seeds of *impatiens novette* (Fig. 2).

Dormancy induction in nondormant lettuce seeds by GA synthesis inhibitors and its relief by GA_{4+7} irradiation, or moist-chilling. Dormancy was induced in nondormant 'Montello' lettuce seeds soaked in the dark for 24 h in 50 μM tetcyclacis, as the seeds failed to germinate upon washing and transfer to water in the dark. The induced dormancy was released by irradiation (Fig. 3A) or by GA_{4+7} (Fig. 3B). A 24-h dark soak in 50 or 100 μM ABA failed to induce dormancy as the seeds germinated readily on transfer to water in both light and darkness. The time needed for complete release of dormancy varied among the cultivars (not shown). Dormancy induced by 5 or 50 μM tetcyclacis was released to a large extent in various cultivars by irradiation (Table 1), a soak in 1mM GA_{4+7} (Table 2), or by moist-chilling at 5C (Table 3). Following the induction of dormancy with tetcyclacis, lettuce seeds, soaked for

up to 10 days in water at 25C, or dried back at 25C by forced air to original weight, responded fully to dormancy releasing treatments (light, GA_{4+7}) (data not shown). Dormancy release resulted in normal seedling development in all cases.

Other GA biosynthesis inhibitors, ancymidol, paclobutrazol, and flurprimidol, also induced dormancy in lettuce seeds (Table 4). As with tetcyclacis, irradiation, GA_{4+7} treatment, and moist-chilling were effective in releasing dormancy.

A progressively greater degree of dormancy was induced in 'Mesa 659' lettuce seeds, with increasing soaking period in 50 μM

Fig. 1. Effect of (A) tetcyclacis and (B) paclobutrazol on germination of 'Mesa 659' lettuce seeds in light and darkness at 25C. Seeds were germinated for 10 days. Bars in (A) and (B) indicate $\text{LSD}_{0.05}$.

Fig. 2. Effect of tetcyclacis on germination of *impatiens novette* seeds in light and darkness at 25C. Seeds were germinated for 10 days. Bar indicates $\text{LSD}_{0.05}$.

Fig. 3. Comparative effects of tetcyclacis (TCY) and ABA on dormancy induction in 'Montello' lettuce seeds and its release by (A) irradiation and (B) GA₄₊₇. After a 24-h presoak in darkness in 50 μ M TCY or 50 μ M ABA, the seeds were washed, dried, and germinated (A) in water in light or darkness and (B) in water or 1 mM GA₄₊₇ in darkness at 25C. Legends in A: germinated in water in light (□), germinated in water in dark (■), presoaked in TCY then germinated in water in light (○), presoaked in TCY then germinated in water in dark (●), presoaked in ABA then germinated in water in light (Δ), presoaked in ABA then germinated in water in dark (▲). Legends in B: germinated in water (■), germinated in GA (●), presoaked in TCY then germinated in water (○), presoaked in TCY then germinated in GA (Δ), presoaked in ABA then germinated in water (▲), presoaked in ABA then germinated in GA (Δ). Bars indicate LSD_{0.05}.

Table 1. Release of tetcyclacis-induced (TCY) dormancy^z in seeds of lettuce cultivars by white light.

Cultivar	TCY (μ M)	Germination (%) ^y	
		Dark	Light
Grand Rapids	5 μ M	0 (35) ^x	98 (99)
Emperor	50 μ M	6 (99)	99 (100)
Ithaca	50 μ M	13 (97)	96 (98)
Garnet	5 μ M	0 (0)	85 (99)
Montello	50 μ M	5 (96)	94 (98)
Empress	50 μ M	0 (97)	97 (97)
Mesa 659	50 μ M	0 (98)	93 (98)
LSD _{0.05}		3 (2)	4 (2)

^zDormancy was induced by a 24-h dark soak in 5 or 50 μ M TCY at 25C, followed by washing and drying in darkness at the same temperature.

^yDormant seeds were germinated in water at 25C in light or darkness for 10 days.

^xData in parentheses are germination percentages of seeds not treated with TCY.

Table 2. Release of tetcyclacis induced dormancy^z in seeds of lettuce cultivars by 1mM GA₄₊₇.

Cultivar	Dark germination (%) ^y	
	-GA ₄₊₇	+ GA ₄₊₇
Grand Rapids (5 μ M)	0	98
Emperor (50 μ M)	3	100
Ithaca (50 μ M)	6	97
Garnet (5 μ M)	0	93
Montello (50 μ M)	6	96
Empress (50 μ M)	0	86
Mesa 659 (50 μ M)	0	88
LSD _{0.05}	2	3

^zDormancy induction by TCY, washing and drying of seeds were as in Table 1.

^yDormant seeds were germinated in water or 1mM GA₄₊₇ at 25C in darkness for 14 days.

Table 3. Release of tetcyclacis induced dormancy^z in seeds of lettuce cultivars by moist-chilling.

Cultivar	Dark germination (%)	
	Dormant (before chilling)	Chilled ^y
Grand Rapids (6 days) ^x	0	90
Emperor (2 days)	6	92
Ithaca (10 days)	3	90
Garnet (6 days)	0	96
Montello (10 days)	6	85
Empress (6 days)	0	97
Mesa 659 (5 days)	0	93
LSD _{0.05}	2	4

^zDormancy induction, concentration of tetcyclacis used for each cultivar, washing and drying were as in Table 1.

^yDormant seeds were moist-chilled at 5C in darkness and germinated in water at 25C for 7 days.

^xNumbers in parentheses for each cultivar are days moist-chilled at 5C.

Table 4. Induction of dormancy^z in 'Mesa 659' lettuce seeds by paclobutrazol (PL), ancymidol (AL), and flurprimidol (FP) and its release by GA₄₊₇, irradiance, and moist-chilling.

Treatment	Germination (%) ^y			
	Dark	Dark + GA	Light	Chilled
AL, 200 μ M	2	84	96	Not tested
PB, 100 μ M	4	100	100	98
FP, 100 μ M	4	90	98	Not tested
LSD _{0.05}	1	3	2	Not tested

^zDormancy induction, washing and drying were as in Table 1.

^yDormant seeds were germinated for 10 days at 25C in water in darkness, in 1 mM GA₄₊₇ in darkness, in water in light, and in water in darkness after 5 days moist-chilling at 5C.

Fig. 4. Effect of presoaking 'Mesa 659' lettuce seeds in tetacyclacis solution for various periods on induction of dormancy. Seeds were soaked in darkness at 25C in 50 μ M tetacyclacis for 0 to 24 h or in 50 μ M ABA for 24 h, washed, dried, and germinated in water at 25C in darkness. Bar indicates LSD_{0.05}.

Fig. 5. Effectiveness of tetacyclacis (TCY) and ABA to induce dormancy in 'Emperor' lettuce seeds following presoaking of seeds in water for various periods. Seeds were soaked in water for up to 10 h in darkness at 25C, transferred to 50 μ M TCY or 50 μ M ABA for 24 h in darkness at 25C, and then washed, and germinated in water at 25C in darkness for 10 days. Bar indicates LSD_{0.05}.

tetacyclacis as fewer seeds germinated upon transfer to water (Fig. 4). After a 10-h soak, about 80% seeds were rendered dormant, and after 16- to 24-h dormancy was induced in 95% to 100% seeds. Unlike tetacyclacis, a soak in 50 μ M ABA for 24 h failed to induce dormancy as the seeds germinated readily upon washing.

To determine if any discrete point during imbibition was critical for inducing dormancy, tetacyclacis was applied to lettuce seeds at various times of soaking in water. Dormancy was induced completely by tetacyclacis applied to seeds presoaked for up to 4 h in water and partly in seeds presoaked for 6 h (Fig. 5). After 8-h soak nearly all seeds germinated, implying that GA synthesized during this period was critical to preventing dormancy induction by tetacyclacis. At no time during the 10-h presoak was ABA able to

Table 5. Comparative effects of tetacyclacis (TCY) and ABA on dormancy induction in 'Emperor' lettuce seeds and its prevention by GA₄₊₇.

Treatment ^z	Germination/100 seeds ^y		Total germination (%)
	During initial soak	After washing	
TCY	2	4	6
TCY + GA ₄₊₇	85	12	97
ABA	0	90	90
ABA + GA ₄₊₇	0	98	98
LSD _{0.05}	4	6	3

^zSeeds were soaked at 25C for 24 h in darkness in 50 μ M TCY and 50 μ M ABA, with or without 1 mM GA₄₊₇.

^ySeeds that germinated during initial 24-h soak were counted. Remaining nongerminated seeds were washed, dried, and germinated in water for 48 h at 25C in darkness.

Table 6. Comparative effects of tetacyclacis (TCY) and ABA on dormancy induction in 'Mesa 659' lettuce seeds during osmotic soak, its prevention by simultaneous application of GA₄₊₇, and the release of TCY-induced dormancy by GA₄₊₇.

Osmotic soak ^z	Germination (%) ^y	
	Water	GA ₄₊₇
PEG	96	99
PEG + TCY	6	99
PEG + ABA	90	98
PEG + TCY + GA ₄₊₇	98	97
LSD _{0.05}	4	1

^zTwo-day osmotic soak in darkness at 25C in -1.2 MPa PEG solution, with or without 50 μ M TCY, 50 μ M ABA, and 50 μ M TCY plus 1 mM GA₄₊₇.

^yAfter osmotic soak, seeds were washed and germinated in water or 1 mM GA₄₊₇ in darkness for 6 days at 25C.

Table 7. Comparative effects of tetacyclacis (TCY) and ABA on dormancy induction in 'Mesa 659' lettuce seeds at 5C, its prevention by GA₄₊₇, and the release of induced dormancy by GA₄₊₇ or irradiation.

Soak at 5C ^z	% Germination ^y		
	Dark	Light	Dark + GA ₄₊₇
TCY	9	95	95
ABA	97	98	98
TCY + GA ₄₊₇	85	98	96
LSD _{0.05}	4	2	2

^zSeeds were soaked for 24 h at 5C in darkness with 50 μ M TCY, 50 μ M ABA, and 50 μ M TCY plus 1 mM GA₄₊₇.

^yFollowing the 5C soak treatment, seeds were washed in darkness and germinated at 25C in water in darkness, in water in light, or in 1 mM GA₄₊₇ in darkness for 6 days.

induce dormancy.

Prevention of dormancy induction by GA₄₊₇ in lettuce seeds under favorable and unfavorable conditions. In 'Emperor' lettuce seeds soaked simultaneously in tetacyclacis and GA₄₊₇ for 24 h at 25C (a favorable condition for germination), the dormancy induction was prevented by GA₄₊₇, as the majority of seeds (85%) germinated during the initial soak, and a few after washing (Table 5). Dormancy was not induced by ABA even though it inhibited germination during the initial soak. Tetacyclacis also induced dormancy in 'Mesa 659' lettuce seeds during an osmotic (Table 6) or 5C (Table 7) soak (unfavorable conditions for germination), and the dormancy induction was prevented by GA₄₊₇; in these cases, the counteractive effect of simultaneous application of GA₄₊₇ and

Table 8. Effect of fluridone (FL) applied in the presence or absence of ABA or tetacyclacis (TCY) during thermodormancy induction in 'Mesa 659' and 'Emperor' lettuce seeds.

Soak at 35C ^z	Germination at 25C (%) ^y	
	Mesa 659	Emperor
Untreated (0-h soak at 35C)	99	98
Water	3	13
ABA	7	4
FL	99	96
ABA + FL	97	100
TCY	---	2
TCY + FL	---	98
LSD _{0.05}	4	6

^zSeeds were soaked for 48 h in darkness at 35C in 22.5 μ M FL, 100 μ M ABA, 50 μ M TCY, or combinations thereof.

^yAfter 35C soak, seeds were washed and germinated in water for 6 days in darkness.

Table 9. Dormancy induction by tetacyclacis (TCY) in seeds of pepper, tomato, onion, carrot, and the effectiveness of GA₄₊₇ and light to release the dormancy.

Seed/TCY soak ^z	Germination (%) ^y		
	Dark	Dark + GA	Light
Tomato, 50 μ M	8 (98) ^x	96	6 (97)
Pepper, 50 μ M	2 (96)	98	3 (95)
Carrot, 10 μ M	0 (85)	88	0 (84)
Onion, 50 μ M	35 (96)	95	40 (93)
Celery, 10 μ M	0 (0)	10	2 (76)
Impatiens, 50 μ M	0 (75)	---	80 (81)
LSD _{0.05}	7 (6)	5	6 (7)

^zSeeds were soaked in TCY solutions (10 or 50 μ M) at 25C (except celery which was soaked at 20C) for 24 h in darkness, followed by washing and drying as in Table 1.

^yDormant seeds were germinated at 25C (except celery seeds which were germinated at 20C) in water in darkness, in water in light, and in 1 mM GA₄₊₇ in darkness for 10 days.

^xData in parentheses are germination percentages of seeds not treated with TCY.

tetacyclacis was expressed after a transfer of the seeds to water at 25C. The data also show that dormancy induced by tetacyclacis can be released by irradiation or GA₄₊₇ applied after the removal of tetacyclacis by washing. Unlike tetacyclacis, ABA failed to induce dormancy when applied during favorable (25C) or unfavorable conditions (reduced ψ or at 5C) for germination.

Effect of fluridone on thermodormancy induction in lettuce seeds. Thermodormancy was induced in both 'Mesa' and 'Emperor' lettuce seeds soaked at 35C for 48 h in darkness, as the seeds failed to germinate upon transfer to 25C in darkness (Table 8). Addition of fluridone during the 48-h soak at 35C prevented the thermodormancy induction in both cultivars. However, neither ABA nor tetacyclacis counteracted the dormancy-preventing effect of fluridone.

Dormancy induction and release in seeds besides lettuce. Dormancy was also induced in tomato, pepper, carrot, onion, impatiens, and celery seeds to varying degrees with tetacyclacis (Table 9) as well as other GA synthesis inhibitors (not shown). Impatiens seeds behaved like lettuce as dormancy induced by 50 μ M tetacyclacis was released on exposure to light. Unlike lettuce, dormancy induced in carrot, tomato, pepper, and onion seeds was not released by either light or by moist-chilling at 5C (data not shown), but was released by GA₄₊₇. Celery seeds used were

dormant in darkness but not in light. Tetacyclacis intensified the dormancy as the responsiveness of seeds to light or GA₄₊₇ (untreated celery seeds germinated 80% in 1mM GA₄₊₇ in darkness) was reduced.

Discussion

The studies show that GA biosynthesis inhibitors induce dormancy in seeds of several genera and species. Dormancy is released by GA₄₊₇, suggesting that the amount of GA synthesized during soaking may determine dormancy induction and release. That dormancy induced by GA synthesis inhibitors could be released also by light and moist-chilling (e.g., in lettuce seeds), factors that mimic the GA effect, further supports the involvement of GA in dormancy prevention and release. Tetacyclacis, paclobutrazol, ancymidol, and flurprimidol inhibit oxidative steps from *ent*-kaurene to *ent*-kaurenoic acid in the gibberellin pathway and are known to retard cellular elongation (Rademacher, 1991). Thus, the dormancy-inducing action of these inhibitors may be related to a block of GA-mediated processes controlling cellular elongation.

The inhibitors, tetacyclacis and paclobutrazol, were more effective in darkness than in light in inhibiting germination and inducing dormancy in lettuce seeds. Also, the dormancy-inducing effect of these inhibitors was reversed by GA, irradiation, and moist-chilling. Synthesis of GA may occur during or subsequent to irradiation and moist-chilling. It is also possible that the sensitivity of the receptor sites to GA, modulating induction, and release of dormancy, is enhanced by moist-chilling and light in a similar fashion. In either case, the need for GA for dormancy release would be diminished. Moist-chilling and GA treatments are reported to enhance the sensitivity of seeds to light (Rethy et al., 1987; VanDerWoude, 1989). Also, the sensitivity of lettuce seeds to GA decreases as the temperature increases, requiring higher GA concentrations to release dormancy or promote germination in darkness (Khan, 1968; Takeba and Matsubara, 1979). Thus, the GA requirement for the prevention and release of dormancy may be governed by changes in seed environment.

Unlike tetacyclacis, ABA failed to induce dormancy in lettuce seeds soaked in the dark at 5C, 25C, or during low ψ seed-hydration treatment. Noninduction of dormancy by ABA cannot be attributed to lack of ABA penetration because ABA strongly inhibits seed germination (Khan, 1968; see also Table 5). Only when ABA is removed by washing is its effect nullified or considerably reduced. Compared to a more permanent effect of GA synthesis inhibitors, effect of ABA is transient and probably not directly related to dormancy induction.

Although fluridone, which is regarded as indirectly influencing ABA production (Le Page-Degivry and Garelo, 1992), prevented thermodormancy induction in lettuce seeds soaked at 35C, the dormancy-preventing effect of fluridone was not counteracted by ABA. It is not known if ABA is produced during thermo-inhibition and that fluridone action is related to a decrease in ABA level. There are several recent reports correlating increased ABA production with its inhibitory effect on germination or growth processes (Karssen et al., 1989; Le Page-Degivry and Garelo, 1992). However, these do not constitute definitive evidence for regarding ABA as a dormancy factor. In immature embryos of sunflower, fluridone treatment decreased the level of ABA and improved the germination, but ABA treatment itself was ineffective in inducing dormancy as the embryos germinated upon removal of ABA (Le Page-Degivry and Garelo, 1992). In addition to inhibiting ABA production, fluridone appears to interfere with tetacyclacis action

(Table 8). Thus, considerable caution is needed in interpreting results using fluridone.

Gibberellic acid appears to be synthesized quite early during 25C soak of lettuce seeds. When 'Mesa 659' lettuce seeds were soaked directly in tetcyclacis, nearly 80% of the seeds became dormant after 10 h soak (Fig. 4). When tetcyclacis was applied following different periods of water soak, the seeds escaped from the dormancy-inducing effect of tetcyclacis at $\approx$ 8 h (Fig. 5). Thus, a period of 8 to 10 h is critical for inducing dormancy and can be regarded as the dormancy-sensitive phase of imbibition. During this period, GA is probably synthesized, allowing the seed to prevent dormancy induction. As ABA did not induce dormancy in seeds following various periods of water presoak, its action might be unrelated to dormancy induction.

Previous studies with GA synthesis inhibitors were conducted in the context of germination (radicle protrusion). No serious attempts have been made to separate germination inhibition from dormancy induction and dormancy release or prevention from germination promotion. There is sufficient documentation that dormancy induction and release are reversible processes and can operate independently of germination (usually radicle protrusion), which is irreversible (Khan and Karssen, 1980; Khan and Zeng, 1985; Khan and Andreoli, 1992). The release or prevention of dormancy by GA or other factors, such as respiratory inhibitors, moist-chilling, and irradiation, need not manifest itself in actual germination if conditions are not right for germination (Khan and Zeng, 1985; Khan and Andreoli, 1992, see also Table 6 and 7). Thus, it seems that there is no need for GA, irradiation, or moist-chilling for germination of seeds (such as lettuce) once the dormancy release has been accomplished or its induction prevented. These seeds will germinate readily in darkness in absence of GA or irradiation under suitable germination conditions.

The mechanism of dormancy induction by GA biosynthesis inhibitors may differ in different seeds. In lettuce and perhaps other light-requiring seeds, changes in embryo growth potential may be associated with dormancy induction and release (Khan and Samimy, 1982). Dormancy prevention or release may be mediated by GA-stimulated activities of hydrolytic enzymes resulting in the production of osmotically active substances that lower the cellular ψ , improve water uptake, and increase embryo growth potential.

In tomato, pepper, carrot, and onion seeds, dormancy was induced equally well in light and darkness by GA synthesis inhibitors. The state of the endosperm surrounding the embryo seems to determine dormancy in pepper, tomato, and carrot. The GA-deficient mutant seeds of tomato, lacking the ability to synthesize GA, fail to produce endo- β -mannanase and other enzymes needed for the removal of endosperm barrier essential for germination (Groot et al., 1988). A similar mechanism might operate in pepper seeds (Watkins et al., 1985).

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