Seed Dormancy in Red Rice[1]

VI. MONOCARBOXYLIC ACIDS: A NEW CLASS OF pH-DEPENDENT GERMINATION STIMULANTS

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ABSTRACT

The weak acid character of many previously identified, but otherwise chemically dissimilar, dormancy-breaking compounds may contribute to their physiological activity. To test this idea, short chain monocarboxylic acids of one to six carbons, for which no previous reports of such activity exist, were incubated with dormant, dehulled red rice (*Oryza sativa*) seeds. Greater than 90% germination was observed after 24 hours of imbibition with 19 millimolar formic, 53 millimolar acetic, 20 millimolar propionic, 28 millimolar butyric, 20 millimolar valeric, or 16 millimolar caproic acid followed by 7 to 14 days incubation on water at 30°C. Dormancy-breaking activity was pH-dependent. Incubation medium pH values that favored formation of the protonated species resulted in the highest germination percentages. There was no promotive effect of medium pH itself in the range of 3 to 7. In contrast, germination of intact seeds was less than 40% in the presence of 55 millimolar monocarboxylic acids at pH 3, unless seeds were partially dry-afterripened. The pH-dependent activity of these acids was maintained during afterripening of intact seeds. The results are consistent with the idea that the dissociable proton of weak acids is responsible for their dormancy-breaking activity. Many other weak acids may break seed dormancy but have been overlooked due to the rigid pH dependence necessary for activity.

The pH of the incubation medium modulates the dormancy-breaking activity of gibberellic acid (8, 13, 21, 28, 30, 31), nitrite (9, 10), azide, cyanide, and hydroxylamine (11). Substantial germination percentages are obtained under conditions favoring the uncharged form of each of these compounds in solution. The weak acid character of many previously identified, but otherwise chemically dissimilar, dormancy-breaking compounds (6) may contribute to their physiological activity. Therefore, it is possible that other dissociable compounds break seed dormancy but have not been examined under the proper pH conditions. To evaluate this hypothesis, monocarboxylic acids, which have no known promotive role as natural plant regulators, were tested at their pKs under buffered conditions for their ability to break the dormancy of dehulled red rice seeds. In this report, we demonstrate that acids of one to six carbons are highly active pH-dependent dormancy-breaking agents. Further evidence, consist-

ent with the concept that dormancy-breaking activity is due to the neutral form of each substance rather than a general effect of the acidity of the incubation medium, is provided.

MATERIALS AND METHODS

Mature, awnless, strawhulled red rice seeds (*Oryza sativa*) were obtained at the South Farm, Rice Research Station, Crowley, LA in 1985. Harvesting, processing, and storage procedures used were those previously described (9). Air-dried seeds were stored at −15°C until use.

Germination tests were performed using the system described by Cohn and Hughes (11). Seeds were incubated in buffered aqueous solutions of formic (pK=3.75), acetic (pK=4.76), propionic (pK=4.87), butyric (pK=4.82), valeric (pK=4.82), or caproic (pK=4.83) acid for 24 h. Germination was scored during a subsequent 7- or 14-d incubation on water. The pH of each test solution was recorded after incubation to check for sufficient buffering capacity during imbibition. All incubations were performed at 30°C in darkness. Each treatment consisted of five replications, and experiments were repeated at least three times. All chemicals employed were reagent grade and stored at room temperature. Monocarboxylic acid solutions were buffered with 25 mM citrate-phosphate and prepared fresh daily. Dilute HCl or NaOH were used to adjust the pH of test solutions. When required, intact seeds were dehulled by hand just prior to treatments. For experiments investigating the effect of dry-afterripening at 30°C upon chemical response of intact seeds, the procedure of Cohn and Hughes (11) was employed.

RESULTS

At their respective pK values, formic, acetic, propionic, butyric, valeric, and caproic acids broke dormancy of dehulled red rice. Maximum germination was observed after 24 h imbibition in each acid at 16 to 53 mM (Figs. 1, 2). Kinetics of germination were especially rapid for seeds exposed to 20 mM propionic acid during imbibition with greater than 95% of the population showing radicle protrusion after 2 d on water. Germination kinetics for seeds imbibed in formic, acetic, and butyric acid were also rapid with almost complete germination observed in 3 d. All acid treatments except valeric and caproic exhibited normal, healthy seedling growth at concentrations resulting in greater than 90% germination. Seeds exposed to valeric or caproic acid showed delayed germination as well as stunted growth (especially radicle injury) at concentrations greater than 12 mM (Fig. 2). Buffer controls at each pH germinated 15% or less (Figs. 1, 2).

For dehulled seeds, the dormancy-breaking action of each monocarboxylic acid was dependent upon the pH of the incubation medium. With constant added amounts of each substance, increasing the pH to favor the dissociated form of each

acid reduced germination to control levels at pH 6 and 7 (Table I). The dependence of dormancy-breaking activity upon the presence of the undissociated form of each acid could also be discerned by varying the amount of each substance added at constant pH and plotting germination *versus* the undissociated acid concentration in the medium as calculated from the Henderson-Hasselbalch equation. Propionic, butyric (Fig. 3), formic, and acetic (data not shown) acids showed similar germination responses for the same concentrations of undissociated acid irrespective of incubation medium pH. This was generally true
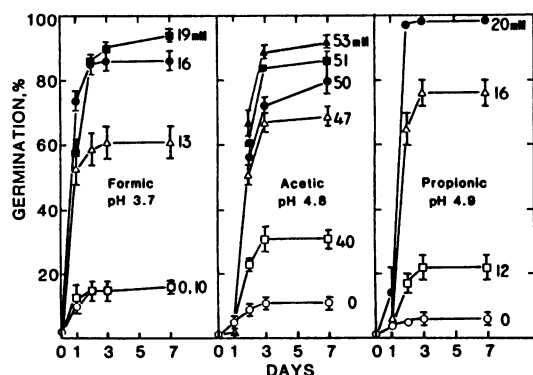

FIG. 1. Effect of formic, acetic, and propionic acids (undissociated plus dissociated forms) on germination of dehulled red rice. Seeds were in contact with each compound at the indicated pH (pK of each acid) for 24 h and subsequently incubated on H_2O for 0 to 7 d. Vertical bars represent the SE.
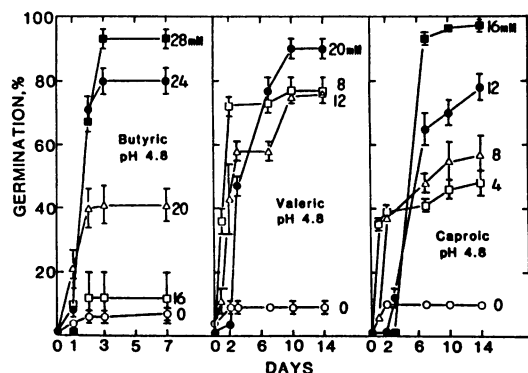

FIG. 2. Effect of butyric, valeric, and caproic acids (undissociated plus dissociated forms) on germination of dehulled red rice. Seeds were in contact with each compound at the indicated pH (pK of each acid) for 24 h and subsequently incubated on H_2O for 0 to 7 d (butyric) or 0 to 14 d (valeric and caproic). Vertical bars represent the SE.

for caproic acid (Fig. 3) and valeric acid (not shown), but significant variability was observed at one data point (5–6 mM undissociated form) for each of these two acids.

Intact seeds germinated up to 40% after contact with monocarboxylic acids at pH 3 but not at pH 7 (Table II, d 0). Dry-afterripening of intact seeds also increased germination in response to these weak acids at pH 3 but not at pH 7 (Table II). Incubation of intact seeds with monocarboxylic acids prior to afterripening under optimum conditions for germination of dehulled seeds (Figs. 1, 2) did not stimulate germination above buffer control levels (data not shown).

DISCUSSION

Populations of dormant, dehulled red rice seeds will germinate almost completely after 24 h of contact with monocarboxylic acids (C1–C6) in the millimolar range. Acids of one, three, or five carbons were required at about 20 mM for 90% germination. Acids containing an even number of carbon atoms showed increased activity with increasing chain length (53, 28, and 16 mM for acetic, butyric, and caproic, respectively, for 90% germination) (Figs. 1, 2). Dormancy-breaking activity of all compounds was pH dependent. Activity was observed under conditions that favored the formation of the protonated form of each chemical (Fig. 3; Table I). These data are consistent with previous work on other dormancy-breaking compounds that are weak acids (8–11, 13, 21, 28, 30, 31). Adkins *et al.* (3) have also shown that seed dormancy in dehulled, partially afterripened wild oats (*Avena fatua*) can be broken by organic acids of glycolysis and the Krebs cycle. However, activity with these intermediates was observed at pH 2.6 as well as with 1 to 10 mM HCl controls. Further experiments with their system at more moderate pH values where control germination values are low should clarify the significance of their experiments.

Increased sensitivity of intact seeds to dormancy-breaking compounds was observed during afterripening (Table II) confirming previous reports (1, 2, 9–11, 15). These data suggested that increased hull permeability to applied chemicals may occur during dry-afterripening. However, in contrast to inorganic dormancy-breaking compounds (9–11), the pH dependence of monocarboxylic acid activity in red rice was not relieved by afterripening.

While the growth-promoting activity of monocarboxylic acids in this study appears to be a surprising result based upon the inhibitory responses generally associated with short chain fatty acids (5, 7, 12, 14, 17, 22, 24, 27, 33, 34), a significant number of previously identified, dormancy-breaking chemicals (6) are also weak acids as well as growth inhibitors in other biological systems. The diverse chemical structures of these compounds have made it difficult to arrive at a unified hypothesis as to how

Table I. *Effect of Incubation Medium pH upon Germination of Dehulled Red Rice in Response to Monocarboxylic Acids*

Treatment[a]	Medium pH					
	3.75	4.0	4.8	5.0	6.0	7.0
mM			germination (%)			
Formic (19)	94 ± 2[b]	60 ± 10		9 ± 2	3 ± 1	6 ± 1
Acetic (53)			92 ± 2	25 ± 3	4 ± 2	3 ± 2
Propionic (20)			98 ± 1	89 ± 3	4 ± 1	6 ± 1
Butyric (28)			93 ± 2	79 ± 3	3 ± 2	4 ± 1
Valeric (20)		91 ± 2	90 ± 2	69 ± 4	13 ± 3	11 ± 3
Caproic (16)			97 ± 2	76 ± 4	28 ± 3	3 ± 1
Control[c]	16 ± 2	9 ± 3	9 ± 1	13 ± 2	6 ± 2	5 ± 2

[a] Chemical treatments: 24 h contact with each organic acid (mM undissociated plus dissociated forms) followed by 7 d on H_2O, except for seeds treated with valeric and caproic acids which were incubated on H_2O for 14 d. [b] Mean ± SE. [c] 24 h contact 25 mM citrate-phosphate buffer followed by 14 d on H_2O.
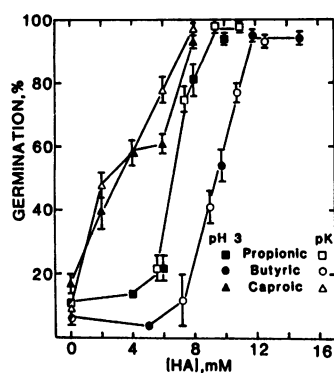
FIG. 3. Effect of undissociated propionic, butyric, and caproic acid concentration at constant pH on germination of dehulled red rice. Seeds were in contact with each compound for 24 h at either the pK of each acid or pH 3 and subsequently incubated on H_2O for 7 d (propionic and butyric) or 14 d (caproic). Vertical bars represent the SE.

Table II. *Effect of Dry-Afterripening on Germination of Intact Red Rice in Response to Monocarboxylic Acids*

Treatment[a]	pH	Afterripening (d)		
		0	3	7
		germination (%)		
Control[b]	3	0 ± 0[c]	6 ± 3	27 ± 2
	7	0 ± 0	2 ± 1	21 ± 3
Formic	3	38 ± 2	64 ± 3	80 ± 2
	7	2 ± 1	1 ± 1	30 ± 3
Acetic	3	29 ± 6	49 ± 3	81 ± 1
	7	1 ± 1	5 ± 2	25 ± 4
Propionic	3	24 ± 4	44 ± 3	68 ± 4
	7	4 ± 1	13 ± 4	26 ± 4
Butyric	3	4 ± 1	21 ± 3	53 ± 3
	7	1 ± 1	1 ± 1	31 ± 4

[a] Chemical treatments: 24 h contact with 55 mM organic acid (undissociated plus dissociated forms) and 25 mM citrate-phosphate buffer followed by 7 d on H_2O.   [b] 24 h contact with 25 mM citrate-phosphate buffer followed by 7 d on H_2O.   [c] Mean ± SE.

they alleviate the dormant state.

However, while these weak acids contain various anionic components, they all possess a dissociable proton. The incubation conditions reported in this and other studies would favor movement of the undissociated form of weak acids into seeds, where it is assumed that dissociation would occur due to a more neutral pH environment. Therefore, it is reasonable to speculate that dormancy is broken by weak acids by facilitating proton transport into seeds. Such a stressful, acid loading of the tissue may activate proton pumping (4, 16, 26, 29, 32) in a manner complementary to the working hypothesis for dormancy-breaking action of fusicoccin (25). Alternatively, acid loading may reduce the internal pH (20, 23) or modify membrane structure (18, 19, 23) sufficiently to allow diffusion of inhibitors out of the seeds.

In summary, a new group of dormancy-breaking compounds has been identified that have weak acid properties similar to many other germination stimulants (8–11). The data are consistent with our previous suggestions that activity of weak acids is due to the protonation of these materials rather than a nonspecific effect of the acid milieu upon seeds during incubation (9–11). Implicit in our findings is the possibility that many other compounds with dormancy-breaking activity have been overlooked in previous work because the appropriate controlled incubation medium pH conditions were not employed.

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