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Seed Dormancy in Red Rice[1]

V. RESPONSE TO AZIDE, HYDROXYLAMINE, AND CYANIDE

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ABSTRACT

The activity of NaN_3 (0.5 millimolar), hydroxylamine-HCl (10–18 millimolar), and potassium cyanide (1 millimolar) as dormancy-breaking agents of dehulled red rice (*Oryza sativa*) is pH-dependent such that medium pH values favoring formation of the uncharged chemical species resulted in the highest germination percentages. There was no promotive effect of pH itself in the range of 3 to 10. The minimum contact times for maximum response (≥90% germination) to NaN_3, KCN, and NH_2OH-HCl are 8 hours at pH 4, 24 hours at pH 8, and 72 hours at pH 6 or 7, respectively, for exposure commencing at the start of imbibition. Dehulled seeds, imbibed first in water, show only slightly reduced germination when subsequently transferred to solutions of dormancy-breaking chemicals.

Intact seeds remain dormant in the presence of NaN_3, KCN, or NH_2-OH-HCl unless partially dry-afterripened. The pH dependence of these chemicals is reduced in intact, afterripening seeds.

Azide, cyanide, and hydroxylamine are known dormancy-breaking agents of seeds (2, 3, 7, 8, 10, 11, 20–23). In most cases, only partial germination of a highly viable seed population was observed after chemical treatment. However, in other studies, maximum dormancy-breaking activity of gibberellic acid (17), nitrite (5), and NO_2 (6) was obtained with the incubation medium pH adjusted so that the undissociated form of each chemical was the most prevalent in solution. Considering the importance of solution pH for penetration and/or activity of weak acids and bases in a wide variety of situations (13–16, 19), it has been suggested that the incubation medium pH should be controlled when studying dissociable, dormancy-breaking chemicals (5).

Another common problem is the lack of reproducibility of germination percentages between seed lots of different ages after chemical treatment. Very short periods of dry-afterripening have been shown to increase the sensitivity of red rice to nitrate, nitrite, and nitrogen dioxide (5, 6). Furthermore, in red rice, afterripening diminished the pH dependency necessary for nitrite activity (5). Afterripening-enhanced sensitivity to ethylene (1), nitrite, hydroxylamine (2), azide and cyanide (9) has been reported in other species as well.

It is also fairly well established that extended imbibition of dormant seeds in water results in a loss of sensitivity to subsequently applied dormancy-breaking chemicals (8, 12, 18). Rapid loss of sensitivity to chemicals applied to red rice under these circumstances has been observed for kinetin and nitrite (4, 5).

It was of interest to extend our recent observations concerning the effects of afterripening, H_2O incubation, and incubation medium pH (5, 6) to other dormancy-breaking chemicals, particularly those with neutral and basic pK_a values. In this report, we describe the response of intact and dehulled red rice to azide, cyanide, and hydroxylamine.

MATERIALS AND METHODS

Mature, strawhulled red rice (*Oryza sativa*) was obtained from fields adjacent to the Rice Experiment Station, Crowley, LA in 1982. Harvesting, processing, and storage procedures used were those described previously (5).

Germination tests were conducted in the system described by Cohn and Castle (6). Lots of 20 seeds were placed in 50 ml Erlenmeyer flasks containing 2 layers of Whatman No. 1 filter paper and 2 ml of test solution, buffer, or water as indicated. Flasks were sealed with unautoclaved rubber septum caps. After treatment, seeds were rinsed from flasks, washed copiously with running water, briefly blotted with tissue, and transferred to clean flasks containing filter paper and 2 ml of the required solution. The medium pH of each flask was recorded after incubation to check for sufficient buffering during treatment. All incubations were conducted 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. Test solutions were prepared fresh each day. Dilute HCl or NaOH were used to adjust the pH of test solutions buffered with 100 mM citrate-phosphate (pH 4–8), Tricine (pH 8), or CAPS[2] (pH 9, 10). When required, intact seeds were dehulled by hand just prior to treatments. Following treatments, germination percentages were recorded after a 7 d incubation on water in flasks as described above.

Preincubation in Water. Dehulled seeds were imbibed in water in the above system for 0 to 7 d, transferred to clean flasks (as above) containing the chemical under study, incubated for the time period indicated in the legend of Table I, and transferred to water for 7 d at 30°C.

Afterripening. Dormant, intact seeds were dry-afterripened in sealed glass jars at 30°C for up to 14 d. Following afterripening, intact seeds were incubated in test solutions for the desired time interval in flasks and then transferred to water as described above.

RESULTS

Preliminary experiments indicated that incubations with 1 mM KCN, 0.5 mM NaN_3, and 10 mM NH_2OH-HCl resulted in the

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[2] Abbreviations: CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid.

highest germination percentages of dehulled red rice with minimum inhibition of subsequent seedling growth. Using these concentrations, the minimum contact times required for maximum germination were 8, 24, and 72 h for azide, cyanide, and hydroxylamine, respectively (Fig. 1). The dormancy-breaking action of each chemical applied to dehulled seeds was dependent upon the pH of the incubation medium (Fig. 2). Activity of azide, hydroxylamine, and cyanide was greatest at pH 4, 6 to 7, and 8, respectively. At 10 mM (pH 8) hydroxylamine treatment resulted in only 40% germination. Greater than 90% germination was obtained with 18 to 20 mM hydroxylamine (pH 8). Buffer controls did not germinate significantly at any pH. Also, seeds treated with buffer alone at pH 4 for 8 h, followed by incubation in 0.5 mM azide at pH 7 for 8 h, germinated to the same extent as pH 7-azide-treated seeds without pH 4 preincubation (data not shown). Nongerminating seeds were viable as determined by growth of isolated embryos. Preincubation of dehulled seeds in water for up to 7 d prior to chemical exposure did not dramatically reduce the dormancy-breaking activity of azide, hydroxylamine, or cyanide at optimum pH values (Table I).

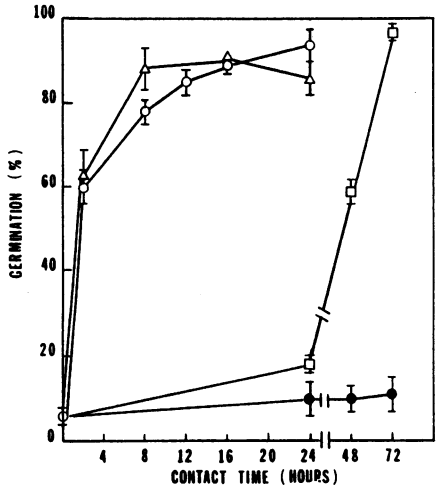

FIG. 1. Effect of contact time with 0.5 mM NaN_3 (△) (pH 4), 10 mM NH_2OH-HCl (□) (pH 7), and 1 mM KCN (○) (pH 8), and buffers (●) (pH 4–8) at 30°C on germination of dormant, dehulled red rice. Incubation following chemical contact was 7 d in H_2O at 30°C. Vertical bars represent the SE.

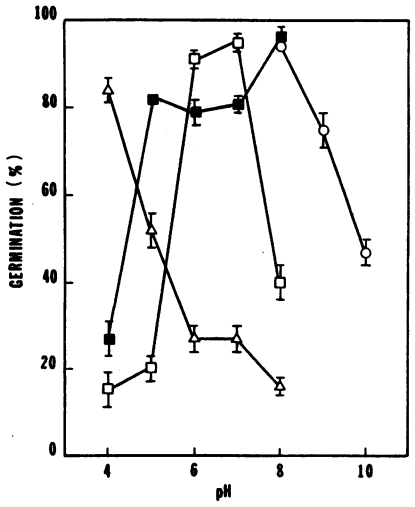

FIG. 2. Effect of incubation medium pH during contact with 0.5 mM NaN_3 (△) (8 h contact), 10 mM (□) and 18 mM (■) NH_2OH-HCl (72 h contact), and 1 mM KCN (○) (24 h contact) at 30°C on the germination of dormant, dehulled red rice. Incubation following chemical contact was 7 d in H_2O at 30°C. Buffer controls germinated 10 ± 5%. Vertical bars represent the SE.

Table I. *Effect of H_2O Preincubation on Germination of Dormant, Dehulled Red Rice in Response to Dormancy-Breaking Chemicals*

Treatment[a]	H_2O Imbibition (days)			
	0	1	3	7
	germination (%)			
NH_2OH-HCl	95 ± 2[b]	91 ± 4	87 ± 5	85 ± 7
NaN_3	86 ± 2	87 ± 4	91 ± 4	90 ± 3
KCN	94 ± 1	93 ± 3	100	
$NaNO_2$	99 ± 1	98 ± 1	89 ± 4	98 ± 1
H_2O	6 ± 3			9 ± 2

[a] Chemical treatments: NH_2OH-HCl: 10 mM (pH 7) 72 h; NaN_3: 0.5 mM (pH 4) 24 h; KCN: 0.5 mM (pH 7) 48 h; $NaNO_2$: 10 mM (pH 3) 4 h. [b] Mean ± SE.

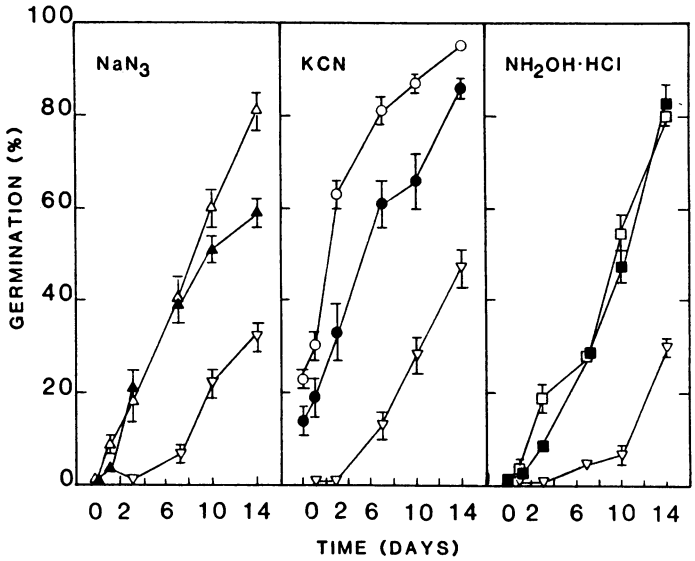

FIG. 3. Effect of NaN_3 (△, pH 4; ▲, pH 6) NH_2OH-HCl (□, pH 6; ■, pH 4), KCN (○, pH 8; ●, pH 11), and buffer controls (▽) on germination of dry-afterripening, intact red rice. Subsequent to afterripening at 30°C, intact seeds were incubated with 0.5 mM NaN_3 (8 h contact), 10 mM NH_2OH-HCl (72 h contact), or 1 mM KCN (24 h contact). Incubation following chemical contact was 7 d in H_2O at 30°C. Vertical bars represent the SE.

In contrast, intact seeds, for the most part, remained dormant after contact with NaN_3, NH_2OH-HCl, or KCN (Fig. 3, time 0) and remained so even with continuous contact for 7 d (data not shown). However, dry-afterripening of intact seeds increased germination in response to these chemicals. Furthermore, the pH requirements which were necessary for activity in previous experiments with dehulled seeds were generally lost as dry-afterripening proceeded (Fig. 3).

DISCUSSION

It has been shown that populations of dormant, dehulled red rice will germinate almost completely and with high reproducibility after contact with appropriate concentrations of azide, hydroxylamine, and cyanide. Contact times required are of short duration (Fig. 1), but hydroxylamine clearly required a longer incubation period than azide or cyanide. This could be due to nonspecific binding of NH_2OH (11).

Significantly, for dehulled seeds, it has been shown that the dormancy-breaking activity of these compounds is pH-depend-

ent. In each case, activity was observed at pH values which favor the formation of the uncharged form of each compound (azide, pK_a = 4.7; hydroxylamine, pK_a = 6.0; cyanide, pK_a = 9.3). These data are consistent with previous work on nitrite, nitrate (5), NO_2 (6), GA_3 (17), and BA (4). The pH dependence of hydroxylamine and cyanide in relation to their pK_a values was important. It further demonstrated that the pH-activity relationship was due to the equilibrium between the charged *versus* uncharged forms of these compounds rather than a general interaction between acidic pH, which might increase activity by weakening the pericarp. Further evidence against the pericarp weakening effect of acid pH as observed by Hsiao *et al.* (12) was the lack of substantial germination of seeds initially incubated in buffer at pH 4 followed by exposure to azide at pH 7. Similar results were obtained for nitrite and nitrate following preincubation with buffer at pH 3 (data not shown).

The consistently reduced response to 10 mM hydroxylamine (pH 8) cannot be accounted for by dissociation to an anionic form. Low germination was probably not caused by significant chemical destruction of hydroxylamine. When NH_2OH solutions incubated at pH 8 for 3 d at 30°C in the absence of seeds were subsequently tested on seeds at pH 6, germination percentages were 89 ± 5%. Increasing the hydroxylamine concentration to 18 mM (Fig. 2) or daily transfer of seeds in 10 mM NH_2OH (pH 8) to fresh medium (data not shown) resulted in maximum germination.

It is possible that the anomalous results obtained with hydroxylamine at pH 8 could be explained by the observation that free NH_2OH reacts with CO_2 to form unstable *n*-hydroxycarbamate at ambient temperatures (24). At basic pH, low levels of free CO_2 would be a potentially limiting factor. However, our attempts to inhibit or accelerate the dormancy-breaking process at pH 6 to 8 by trapping CO_2 in KOH or by adding CO_2 exogenously were without success.

There was little effect of preincubation of dehulled seeds in water for up to 7 d upon dormancy-breaking activity of these compounds. This contrasts with previous reports (5, 8). Reexamination of the procedures used earlier showed that the reported reduction of activity with water preincubation did not occur if nitrite or other compounds (Table I) were applied to the seeds in the presence of buffer. Previous results can be ascribed to the insufficient buffering capacity utilized and the strong pH-dependence of these dormancy-breaking chemicals as well as the probably reduced uptake rate of chemicals after imbibition was completed.

Increased sensitivity of intact seeds to dormancy-breaking chemicals was observed during afterripening which confirmed similar trends reported previously (1, 2, 5, 6, 9). In addition, the pH-dependence for activity in red rice was shown to diminish as afterripening proceeded. These data taken together suggest that a component of the dry-afterripening process should be increased seed permeability to applied chemicals.

In summary, control of incubation medium pH when utilizing dormancy-breaking chemicals which are weak acids or bases may improve the effectiveness and reproducibility of such compounds as dormancy-breaking agents. The interaction between dry-afterripening and enhanced chemical activity may also reconcile the broad, variable range of results obtained when working with seed lots of different ages of the same species.

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