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Seed Dormancy in Red Rice[1]

III. RESPONSE TO NITRITE, NITRATE, AND AMMONIUM IONS

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ABSTRACT

Sodium nitrite at 10 millimolar breaks dormancy of dehulled red rice (*Oryza sativa*). While germination is light independent, low pH conditions (pH 3) are required for maximum response. Water and buffer controls at pH 3 remain dormant. The response to nitrite occurs at 25 and 30°C but is reduced at 20°C, although nondormant seeds germinate readily at this temperature. The contact time for response to nitrite is less than 2 h at the start of imbibition. Seeds imbibed first in water show reduced germination when subsequently transferred to nitrite. Dehulled seeds show little or no response to nitrate and ammonium ions.

Intact seeds remain dormant in the presence of nitrite or nitrate unless partially dry-afterripened. The pH dependence of nitrite sensitivity is reduced in intact, afterripening seeds. In highly dormant seeds, vacuum infiltration experiments suggest that the hull restricts uptake of nitrite.

Red rice, an annual species, is a problem weed in rice production. Seeds of red rice shatter at maturity and can remain dormant in soil (4). The conditions that promote and break dormancy in this species are not well understood. Previously, it has been shown that freshly harvested seeds dry-afterripen at ambient temperatures. Both the hull and pericarp have a role in maintenance of primary dormancy in red rice. However, isolated embryos from freshly harvested seeds germinate readily (1). Primary dormancy of dehulled red rice is broken by exogenously applied cytokinins, but there is no effect on intact seeds (2).

Although there are many reports concerning the response of dormant seeds to nitrate, nitrite, and ammonium ions (*e.g.* 5, 11, and references therein), no information is available for red rice. As part of a comprehensive study of primary dormancy in red rice, the effects of these inorganic nitrogen compounds have been evaluated.

MATERIALS AND METHODS

Mature, strawhulled red rice (*Oryza sativa*) was obtained from fields at the Rice Experiment Station, Crowley, LA, in 1979, 1980, and 1981. Seeds were harvested randomly from individual plants by hand-shattering. Moisture content at harvest was 23% on a fresh weight basis. Processing, storage, and germination test procedures used were those previously described (1). Intact seeds were dried at 22°C on open trays for 5 d and then stored in sealed glass jars at −15°C. Seed moisture during frozen storage was 12%. Unless otherwise specified, seeds from the 1980 harvest were used, and germination tests were performed at 30°C for 7 d in dark incubators. Nine-cm-diameter glass Petri dishes containing one sheet of Whatman No. 3 filter paper and 10 ml of test solution were the standard germination system. A double layer of tissue paper was used to cover the seeds to insure uniform hydration. When required, intact seeds were dehulled by hand just prior to treatments. Five replicates of 30 seeds were used for each treatment. Experiments were repeated at least three times. Chemicals used were reagent grade and stored at room temperature. Test solutions were freshly prepared for each experiment. Dilute HCl or NaOH were used to adjust the pH of solutions.

Procedures for experiments regarding the effects of light, dry-afterripening, and vacuum infiltration were those employed previously (2).

Effects of Light. Dehulled seeds were treated with test solutions under a dim, green safelight and enclosed in black cloth bags. Batches of the same seeds were placed in an incubator and continuously illuminated with both fluorescent and incandescent lamps at 60 $\mu E/m^2 \cdot s$.

Effect of Vacuum Infiltration. Intact seeds were vacuum infiltrated for three 5-min periods with the appropriate test solutions (1 ml solution/seed). Seeds were plated on water or acidified nitrite in Petri dishes and incubated for 7 d at 30°C. Seeds were then dehulled by hand. Dehulled seeds were incubated for an additional 7 d at 30°C in water.

Effect of Afterripening. Intact seeds were dry-afterripened in sealed glass jars at 20 or 30°C for up to 14 d. Following the afterripening period, the intact seeds were incubated in nitrite or water for 7 d at 30°C.

RESULTS

Sodium nitrite at 10 mM (pH 3) broke dormancy of dehulled red rice. Sodium nitrate and ammonium chloride were only slightly active at best. Nitrite activity was highly dependent upon the initial pH of the incubation medium (Table I). The initial pH did not influence the response to nitrate or ammonium ions. There was little effect of continuous illumination on the effect of any ions tested except for a slight increase in germination percentage by light with nitrite at pH 7 (Table I). Viability of seeds in all treatments was greater than 98% as measured by germination of isolated embryos (1).

Reducing the incubation temperature to 20 or 25°C did not increase the germination percentage in the presence of $NaNO_3$, NH_4Cl, or water. Sodium nitrite was still effective in breaking dormancy of dehulled red rice at these temperatures although the magnitude of the response was reduced (35% at 20°C; 67% at 25°C). Part of the reduced germination during incubation with nitrite at 20 or 25°C can be accounted for by reduced viability (61% at 20°C; 75% at 25°C). Seed viability was not reduced in

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Table I. *Interaction of Initial pH and Light with Ammonium, Nitrate, and Nitrite Ions (10 mM) in the Per Cent Germination of Dormant, Dehulled Red Rice*

Treatment	pH 3		pH 7		pH 9	
	Light	Dark	Light	Dark	Light	Dark
$NaNO_2$	90 ± 4[a]	92 ± 2	31 ± 3	18 ± 3		
$NaNO_3$	13 ± 3	10± 2	9 ± 1	5 ± 2		
NH_4Cl	9 ± 3	7 ± 3	7 ± 2	4 ± 2	6 ± 2	6 ± 2
H_2O	7 ± 2	5 ± 1	6 ± 1	3 ± 1		

[a] Mean ± SE.

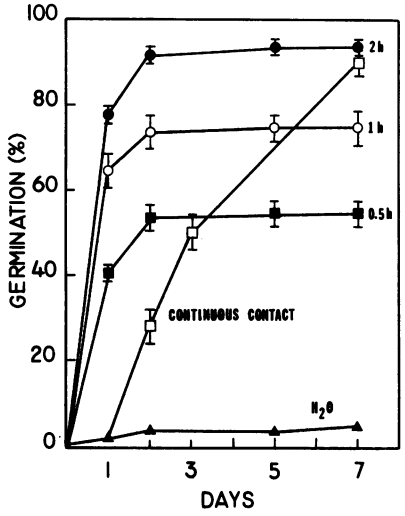

FIG. 1. Effect of incubation time at 30°C on germination of dormant, dehulled red rice in the presence of 10 mM $NaNO_2$ for various contact times (pH 3, initially). Vertical bars represent the SE.

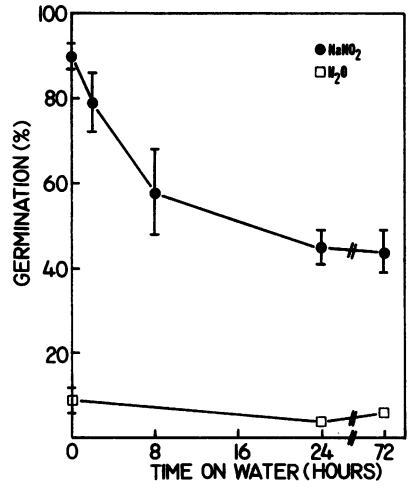

FIG. 2. Effect of preincubation in water on nitrite-stimulated germination of dormant, dehulled red rice. Seeds were incubated for the designated time in water and subsequently transferred to 10 mM $NaNO_2$ (initially pH 3) for an additional 7 d at 30°C in darkness. Vertical bars represent the SE.

the presence of nitrate, NH_4Cl, or water under these conditions. Germination of nondormant seeds is complete within 14 d at either 20 or 25°C.

The response of dehulled seeds to nitrite, nitrate, and ammonium ions was generally consistent in 10 different seed lots collected from different fields and maturing at different times in 1979, 1980, and 1981. All seed lots germinated well (mean: 87 ± 3%) in the presence of 10 mM $NaNO_2$ at pH 3, while response to $NaNO_3$ and NH_4Cl was minimal in most cases (means: 13 ± 2, 10 ± 2, respectively). These data, in contrast to the variable response of barley to gibberellic acid (10), indicate little impact of environmental conditions during seed maturation upon subsequent seed performance. However, the response of one seed lot (SH 79-3) which had been stored for 1.5 years at −15°C differed from other seed lots. Water controls germinated 29% and seeds in $NaNO_3$ germinated 55%. These results suggested that a slight degree of afterripening might influence response to nitrate (see below).

Table II. *Germination of Intact, Dormant Red Rice Following Treatment with Nitrite*

Seeds were vacuum infiltrated three times for 5 min and incubated for 7 d at 30°C. Nongerminating seeds were rinsed with water, dehulled, and incubated in water for 7 d at 30°C. The concentration of nitrite was 10 mM and the initial pH was 3.0.

Vacuum Infiltration Solution	Incubation Medium Intact Seeds	Germination	
		Intact seeds	Dehulled seeds
		%	
None	$NaNO_2$	19 ± 1[a]	13 ± 4
None	H_2O	0 ± 0	4 ± 2
$NaNO_2$	$NaNO_2$	52 ± 3	1 ± 1
$NaNO_2$	H_2O	8 ± 1	7 ± 2
H_2O	$NaNO_2$	38 ± 4	8 ± 3
H_2O	H_2O	0 ± 0	4 ± 2

[a] Mean ± SE.

The responses of dehulled red rice in the presence of 10 mM nitrite at pH 3 were studied in further detail. Germination occurred gradually over a 7-d period in the continuous presence of nitrite (Fig. 1). This was in contrast to the more rapid effects of kinetin on dehulled red rice (2), where almost 70% germination was observed in 2 d.

A very short contact time with acidified nitrite was required to break dormancy of dehulled seeds (Fig. 1). A 2-h exposure at the start of imbibition followed by incubation in water resulted in 90% germination 2 d later. A significant increase in germination was observed with nitrite incubations as short as 0.5 h. This was probably not due to destruction of the pericarp by the acid pH alone because 0.01 N HCl adjusted to pH 3 resulted in only 11% germination after 2 or 24 h of contact followed by incubation in water at 30°C. Also, incubation of seeds in 10 mM phosphate, malate, and citrate at pH 3 for 24 h followed by 7 d on water resulted in less than 10% germination with greater than 90% viability. The pH of these buffers never exceeded 3.4, while the pH of unbuffered nitrite and acidified water controls initially at pH 3 increased to pH 5.2 in 24 h.

If dormant, dehulled seeds were first incubated in water and then transferred to acidified nitrite, germination decreased as the water incubation time was lengthened (Fig. 2). Loss of nitrite sensitivity seemed related to the time course of imbibition, which is complete in 8 to 12 h at 30°C (Cohn, unpublished results). The loss of nitrite sensitivity after water imbibition contrasted with similar experiments with kinetin (2) which showed an apparently imbibition-independent loss of sensitivity. It is not presently clear why this difference between nitrite and kinetin was observed. In both series of experiments, however, approximately 40% of the seeds still germinated after a 3-d water incubation. While we have no explanation as to why sensitivity is not completely lost in the population, the loss of sensitivity may represent a shift to a state of secondary dormancy as has been observed in cocklebur (3).

Intact seeds incubated with acidified nitrite germinated ap-

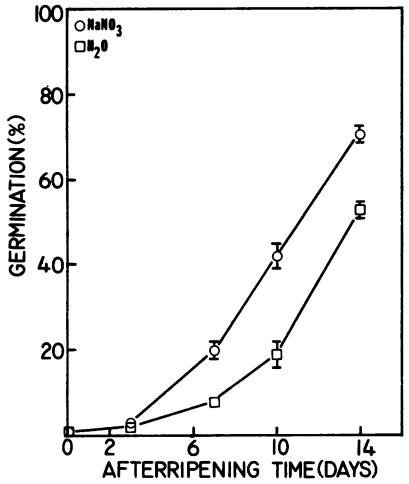

FIG. 3. Effect of $NaNO_3$ treatment on germination of dry-afterripened, intact red rice. Subsequent to afterripening at 30°C, intact seeds were incubated with 10 mM nitrate (initially pH 3) for 7 d at 30°C. Vertical bars represent the SE.

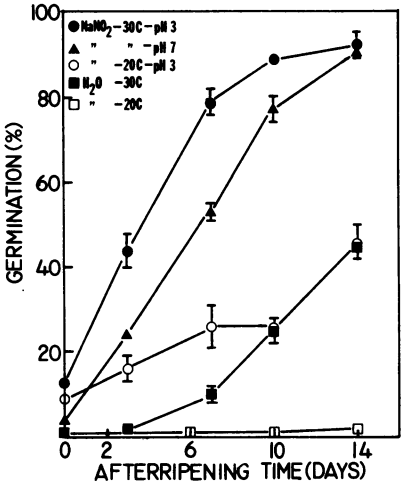

FIG. 4. Effect of $NaNO_2$ treatment on germination of dry-afterripened, intact red rice. Subsequent to afterripening at 20 or 30°C, intact seeds were incubated with 10 mM nitrite at pH 3 or pH 7, as indicated, for 7 d at 30°C. Vertical bars represent the SE.

proximately 20% (Table II). Vacuum infiltration experiments designed to force entry of nitrite into intact seeds indicated that as long as nitrite was present in the incubation medium following infiltration (either with water or nitrite), significant germination of intact seeds occurred (Table II). With water in the incubation medium, infiltration of nitrite or water had little effect on the germination of intact seeds. Subsequent dehulling of vacuum-infiltrated intact seeds resulted in minimal further germination. Total viability of all treatments was 90% or greater. We interpret these results as indicating that the hull restricts nitrite uptake but does not inhibit the dormancy-breaking activity of nitrite after the hull is penetrated. These results contrast with those obtained with kinetin where not only did the hull inhibit uptake but restricted dormancy-breaking activity after vacuum-facilitated penetration (2).

When intact seeds were dry-afterripened at 30°C and subsequently incubated with acidified nitrate, germination increased over that of water-treated controls (Fig. 3). Sensitivity to NH_4Cl did not increase when intact seeds were dry-afterripened (data not shown).

A more dramatic effect of afterripening was observed when intact seeds were incubated in acidified nitrite (Fig. 4). After dry storage at 20°C for 14 d, 45% germination in nitrite was observed compared to 9% germination without afterripening. This increase in nitrite sensitivity occurred without any changes in germination of water controls. Comparable results were obtained when intact seeds were afterripened at 30°C. Furthermore, the acid pH requirement necessary for nitrite activity was observed to diminish as the dry-afterripening period of intact seeds was increased. A similar experiment comparing nitrate at pH 3 *versus* pH 7 showed no differences with afterripening intact seeds (data not shown).

The effect of afterripening on nitrite and nitrate sensitivity contrasts to similar experiments performed with kinetin (2) where afterripening at 30°C had no effect on the response of intact seeds incubated in this cytokinin compared to water controls.

DISCUSSION

It has been demonstrated that the dormancy of dehulled red rice can be broken by nitrite but not by nitrate or ammonium ions. Intact seeds respond most dramatically to nitrite and, to a lesser extent, to nitrate only when partially dry-afterripened. These data generally agree with the trends of results from studies of domesticated rice (nitrite > nitrate > ammonium ions) (11). However, while Roberts (11) concluded that no special precautions need be taken with regard to pH of the incubation medium in the range of pH 3.0 to 8.0, it is clear from our data that attention to medium pH is critical when evaluating nitrite activity.

To our knowledge, this is the first report of the pH dependence of nitrite as a dormancy breaking agent (Table I). Since acidification of nitrite generates the weak acid, nitrous acid (HNO_2) (pK_a = 3.3) (9), these results suggest that the form of nitrogen required for entry and perhaps activity as a dormancy-breaking agent in dehulled seeds is HNO_2.

However, many cases exist in which both nitrite and nitrate are capable of breaking seed dormancy without attention to the pH of the incubation medium (presumably close to pH 7). Results of this sort have been obtained in this study with partially afterripened, intact red rice (Fig. 4). Therefore, it is likely that, in the case of highly dormant red rice seeds, entry of the charged species, NO_2^- and NO_3^-, is restricted by a permeability barrier in the hull which breaks down as the seeds afterripen. The possible existence of this barrier in other species may explain why nitrate alone, in the absence of prechilling, alternating temperatures, or light is relatively ineffective as a dormancy breaking agent (8, 12, 14).

The varying degree of germination obtained when dormant seeds of various species are exposed to nitrite and nitrate may be due to differing states of afterripening inasmuch as many reports in the literature utilize 'dormant' populations of seeds with water controls as high as 30%. Hylton and Bass (6) also observed increased nitrate sensitivity with afterripening in sixweeks fescue. Our results confirm these observations.

Our studies with red rice indicate that more attention should be paid to the pH of the incubation medium when working with nitrite and any other suspected dormancy-breaking agent (7, 13) which can exist as a weak acid. Further, the degree of dormancy-breaking activity of nitrite and nitrate has been shown to be very sensitive to small changes in afterripening which, in some cases, may not be detected by simply using the criteria of low germination of controls in water.

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