Seed Dormancy and Germination of *Echinochloa oryzicola* VASING.: Ethanol as a Dormancy-Breaking Agent*

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Introduction

Echinochloa oryzicola VASING. is a gramineous weed in flooded rice and produces seeds (spikelets) with primary or innate dormancy. The seed dormancy could be artificially broken with aerobic respiration inhibitors such as KCN and NaN_3 , and also with 8-oxyquinoline^{2,7)}. Other than these respiratory inhibitors, anesthetics such as ethanol are often reported to be effective in breaking the seed dormancy of other species^{9,10)}.

Ethanol is the end product of alcohol fermentation, the importance of which in the early germination phase of *E. oryzicola* seeds was indicated in our preceding papers^{11,12)}. Although the dormant seeds were very quiescent in activities of respiratory enzymes and produced a far greater amount of CO_2 than ethanol, the non-dormant seeds rapidly increased in alcohol dehydrogenase activity and produced nearly equimolar amounts of CO_2 and ethanol until the radicle protrusion both in aerobic and anaerobic imbibitions. It thus appeared that the dormant seeds respired to a small extent through the oxidative electron transport chain, whereas the non-dormant seeds respired mainly through alcohol fermentation even during aerobic imbibition.

We have found that ethanol is partially effective in inducing germination of *E. oryzicola*

seeds when used as a solvent for artificial chemical treatments. CHISAKA *et al.* (1967)³⁾ also reported a dormancy-breaking activity of ethanol for this weed seeds. Objective of the experiments reported herein was to clarify the dormancy-breaking efficacy of ethanol and to determine its effect on the water absorption of seeds.

Materials and Methods

Seeds of *Echinochloa oryzicola* with primary dormancy were obtained from the same inbred line reported in the preceding paper¹¹⁾, and stored at -21°C under a dry condition. Upon usage in each experiment the intact seeds (spikelet) were dehulled thoroughly with a sewing needle in order not to injure the pericarp. Germination tests were made by placing intact seeds or dehulled seeds on single Toyo No. 2 filter papers with 5 and 4 ml of distilled water or ethanol solution in 6- and 4.5 cm petri dishes, respectively. In experiments involving test solutions of ethanol and other organic solvents less than 10% (v/v) the test solutions initially prepared at the designated concentrations were placed in the petri dishes and the concentrations were not corrected for vaporization and degradation. Exposure to 80% (v/v) ethanol was carried out by placing seeds in the ethanol solution for designated periods and washing them thoroughly with

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distilled water. In an experiment involving vacuum infiltration seeds were vacuum infiltrated in test solution until complete sedimentation had taken place. Scarification was carried out by placing seeds on sandpaper shaken at 180 rpm for 3 minutes. Germination, assayed by protrusion of the plumule or radicle, was determined after 6 days at 30°C in the light. The number of seeds and repetitions used in each experiment varied and is indicated in the following table. Values obtained in % germination were statistically analyzed, after arc sin transformation, with Duncan's multiple range test (0.5).

In experiments involving determination of water absorption, the seeds treated and placed in petri dishes were removed at intervals from water, blotted with filter papers and weighed promptly.

Results and Discussion

Germination percentages of the dormant seeds treated with ethanol are shown in Table 1. Ethanol was remarkably effective in overcoming the dormancy of dehulled seeds and provided 100% germination at 5% (v/v). Although with no ethanol one among 50 intact seeds of a repetition germinated, virtually no germination was observed when intact seeds were treated with the chemical at up to 10%. The intact seeds vacuum infiltrated showed 61

% germination with 5% ethanol, but 32% even without ethanol. Thus, inefficacy of the chemical on intact seeds appeared to be due to a problem of infiltration, and the vacuum infiltration itself was partially effective. Moreover, high concentration of the chemical had an adverse effect on germination and germination percentage was decreased when dehulled or vacuum infiltrated intact seeds were treated at 10% ethanol.

TAYLORSON and HENDRICKS¹⁰⁾ reported a stimulatory effect on dormant fall panicum (*Panicum dichotomiflorum*) seeds by methanol, acetone, chloroform, ethyl ether and also ethanol. However, on dormant dehulled *E. oryzicola* seeds used in our experiment only methanol showed a stimulatory effect among the alcohols and organic solvents tested (Table 2). Methanol required two-fold greater concentration than ethanol to obtain 100% germination (Table 1 and 2). Table 3 shows the germination percentages of unimbibed dormant dehulled seeds with 80% (v/v) ethanol exposure. Germination percentages linearly increased as the exposure period increased, and 100% germination was obtained by exposing the unimbibed dormant seeds to 80% ethanol for 30 minutes. However, germination percentage declined when the test was conducted with imbibed dormant seeds (Table 4). In the experiment described in Table 4, ungerminating seeds after the 6 day imbibition were tested with 0.5% (w/v) tetrazolium chloride. Both seeds treated with no ethanol and those with ethanol at unimbibing stage were stained completely redly, whereas those treated with ethanol at imbibing stage showed poorly to unstained embryos and a callus-like enlargement of the embryo was observed in many of the seeds.

DENNY (1917) studied the role of lipoidal substances present in the seed coats of various species in limiting the rate of water penetration by treating the seed coats with alcohol, acetone and ether, and reported that these chemicals were effective in increasing the rate

Table 1. Effect of ethanol on germination percentage of dormant *Echinochloa oryzicola* seeds.

Ethanol % (v/v)	% Germination (6 days) ^{a)}		
	Dehulled seeds	Intact seeds	Intact seeds (vac.)
0	0.0 a	0.7 a	32.0 ab
1	8.3 ab	0.0 a	44.0 b
5	100.0 c	0.0 a	61.1 b
10	36.7 b	0.0 a	3.3 a

^{a)} Germination test was conducted with three repetitions, 20/rep. for dehulled seeds and 50/rep. for intact seeds. Values in columns followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Table 2. Effect of alcohols and organic solvents on germination percentage of dormant *E. oryzicola* dehulled seeds.

Chemical ^{a)}	Concentration % (v/v)	% Germination (6 days)	Chemical ^{b)}	Concentration % (v/v)	% Germination ^{c)} (6 days)
Check	—	0.0	Check	—	1.7 a
<i>n</i> -propyl alcohol	1	0.0	Acetone	0.1	0.0 a
	5	0.0		1.0	0.0 a
	10	0.0		5.0	0.0 a
<i>t</i> -Butyl alcohol	1	0.0	Chloroform	0.05	1.7 a
	5	0.0		0.1	0.0 a
	10	0.0		0.5	1.7 a
<i>t</i> -Pentyl alcohol	1	0.0	Methanol	1.0	10.0 b
	5	0.0		5.0	36.7 c
	10	0.0		10.0	100.0 d

^{a)} 10 dehulled seeds/rep., three repetitions.
^{b)} 20 dehulled seeds/rep., three repetitions.
^{c)} Values in columns followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Table 3. Effect of 80% (v/v) ethanol exposure on germination percentage of dormant *E. oryzicola* dehulled seeds.

Exposure (min.)	% Germination (6 days) ^{a)}
0	0.0 a
10	26.7 ab
20	76.7 bc
30	100.0 c

^{a)} 10 dehulled seeds/rep., three repetitions. Values in columns followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Table 4. Differential responsiveness of imbibed and unimbibed dormant *E. oryzicola* dehulled seeds to 80% (v/v) ethanol exposure.

Dehulled seeds	Exposure 30C, 30min.	% Germination ^{a)} (6 days)
Imbibed	Water	0.0 a (+)
	Ethanol (80%)	28.3 b (±)
Unimbibed	Water	1.7 a (+)
	Ethanol (80%)	68.0 c (+)

^{a)} 20 dehulled seeds/rep., three repetitions. Signs, + and ±, in parentheses indicate respectively stained and poorly to unstained embryos found with TTC test. Values in columns followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Fig. 1. Effect of 80% (v/v) ethanol exposure on rate of water absorption of dormant *E. oryzicola* dehulled seeds. 40 seeds/rep., three repetitions.

of water penetration, probably because of their solubilizing effect on the lipoidal substances. The effect of ethanol treatment on water absorption was determined for the dormant *E. oryzicola* seeds. As shown in Figure 1, the pattern of water absorption in scarified dehulled seeds could be divided into three phases as TAKAHASHI⁸⁾ described for rice seeds. The dehulled seeds absorbed very rapidly immediately from the beginning of imbibition to 8 hr (Phase A, the imbibition stage). The water absorption appeared to cease once from 8 to 12 hr (Phase B, the germination state). Germination of the seeds was observed at 31 hr, and the water absorption would then increase further (Phase C, the growth stage). Phase B of seeds with no treatment appeared to last for a prolonged period from 24 hr and no germination was observed. The steeper increase of water content in scarified seeds indicated stimulation of water absorption by scarification. However, the steepness and pattern of water absorption of dormant dehulled seeds treated with 80% ethanol for 30 min were almost identical to those of seeds with no treatment. Phase B was not detected, but germination of some seeds started at 31 hr.

Although difference in % increase from the initial weight was not statistically significant between ethanol-treated and non-treated seeds even at 31 hr (Figure 1), the values of % increase were obtained with a batch of 40 seeds and within this batch of seeds there was a small variation in time when germination was observed. In the experiment described in Table 5, mean fresh weight of a seed was obtained by selecting and weighing individual seeds at a visibly identical stage. Ethanol-treated seeds just protruding plumules weighed 2.27 mg/seed, whereas non-treated seeds with no sign of germination were 2.65 mg/seed at 48 hr. There was no statistical significance between them. Thus, it appeared that the dormancy-breaking effect of ethanol did not relate to water penetration into the seed.

Table 5. Effect of ethanol on the water absorption of dormant *E. oryzicola* dehulled seeds.

Treatment	Mean fresh weight of a seed (mg) ^{c)}	
	A phase ^{a)}	B phase ^{b)}
Check	2.54 a	2.65 a
Ethanol(5%)	2.49 a	2.72 a
Scarified	2.80 b	—

^{a)} 4 hrs in imbibition. Mean weight of a seed was determined in a batch of 25 seeds with three repetitions.

^{b)} 48 hrs in imbibition. Mean weight of a seed was obtained by weighing individual seeds; check (n=55) and ethanol-treated seeds protruding plumules (n=64).

^{c)} Values in columns followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Induction of alcohol dehydrogenase by exogenous supply of ethanol to plant roots was suggested by various authors^{1,4,5)}. In our preceding papers^{11,12)}, we indicated the importance of alcohol fermentation for non-dormant seeds of *E. oryzicola* in germinating both under aerobic and anaerobic conditions. During imbibition the seeds rapidly increased activity of alcohol dehydrogenase and produced nearly equimolar amounts of ethanol and CO₂ until radicle protrusion even under aerobic condition. We proposed that the non-dormant seeds respired mainly through alcohol fermentation even under aerobic condition. Ethanol is the end product of alcohol fermentation. As an explanation of the dormancy-breaking efficacy of ethanol reported herein, it might thus be possible that ethanol applied to the dormant seeds acted as an inducer of alcohol dehydrogenase which catabolised the fermentation. But this may not be the case since the dormant seeds possessed relatively high activity of the enzyme during imbibition, although the seeds produced little ethanol. Therefore, the enzyme operation appeared to be blocked *in vivo* in the dormant seeds.

TAYLORSON and HENDRICKS¹⁰⁾ and HENDRICKS and TAYLORSON⁶⁾ reported the marked dor-

mancy-breaking effect of ethanol and some other anesthetics on seeds of several species including *Echinochloa crus-galli*. From the three facts that : ethanol bypassed a requirement for active phytochrome in enhancing germination in darkness ; and the similarity in action of ethanol, methanol, chloroform, ethyl and acetone on seed germination was in accordance with their lipid solubilities ; and that the seed germination induced by ethanol was prevented by application of pressure > 1 MPa during the period of exposure to the anesthetic, the authors proposed that the dormancy-breaking action of ethanol and other anesthetics depended on modifying a membrane property. Although no direct evidences has not been presented for the mechanism of the dormancy-breaking action of ethanol, it is quite interesting fact that seed dormancy of *E. oryzicola* is released by exogenous application of the chemical which is endogenously produced in non-dormant seeds, but virtually not at all in dormant seeds, prior to a visible sign of germination.

Summary

Dormancy-breaking efficacy on seeds of *Echinochloa oryzicola* VASING. was studied with ethanol, the end product of alcohol fermentation, which is endogenously produced in a large amount in non-dormant seeds prior to the redicle protrusion. Primary dormancy of the seeds was markedly broken when dehulled seeds (fruits) were imbibed in 5 % (v/v) ethanol solution or exposed to 80% ethanol for 30 min. Methanol also showed a dormancy-breaking efficacy, but required higher concentration, among the other alcohols and organic solvents.

No germination was observed when intact seeds (spikelets) were treated with ethanol, probably because of its insufficient penetration, and the intact seeds vacuum infiltrated in 5 % ethanol solution showed relatively high germination percentage. No significant difference

in rate and amount of water absorption was observed between ethanol-treated and non-treated seeds.

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タイヌビエ種子の休眠と発芽

——エタノールの休眠覚醒効果について——

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摘 要

タイヌビエ種子の発芽初期過程において多量のエタノールが幼根突出以前に生成される。この内生的に生成されるエタノールを外生的に休眠種子に与え、その休眠覚醒効果を検討した。エタノールの休眠覚醒効果は顕著であり、除穎した休眠種子（果実）を5% (v/v) のエタノールとともに発芽床（30°C, 明条件）に置床すると、80% エタノールに30分間浸漬した場合とともに100% の発芽率が得られた（Table 1, 3）。他のアルコール類および有機溶媒の休眠覚醒効果とあわせて検討したところ、メタノールに効果が認められたが100% の発芽率を得るためには10% のメタノールが必要であった（Table 2）。

除穎していない休眠種子（小穂）をエタノールで処理しても休眠覚醒効果は認められないが、5% エタノール中でこれを減圧処理すると、61% の発芽率が認められたことから、小穂に対してエタノールが休眠覚醒効果を発揮できない原因は透過性にあると考えられた（Table 1）。また、吸水速度および吸水量においてエタノール処理種子と無処理種子の間に有意な差が認められないことから（Figure 1, Table 5）、休眠覚醒におけるエタノールの作用部位は、果実表層の親油成分の可溶化などによるものでなく、果実の内部に存在することが示唆された。