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Abscisic Acid Levels in the Grain of Wild Rice[1]

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ABSTRACT

Prolonged grain dormancy hinders wild rice (*Zizania palustris* L.) domestication. The purpose of this research was to determine if the inhibitor, abscisic acid (ABA), may be responsible for wild rice grain dormancy.

Nondormant wild rice grains (stored in water at 3 C for 330 days) were dehulled, scarified, and placed in ABA solutions ranging in concentration from 0 to 20 mg/liter for 7 days. Germination decreased as ABA concentrations increased. No germination was obtained at or above 5 mg/liter of ABA. Washing the ABA-treated grains with water did not reverse the inhibition.

Free and conjugated ABA were determined chromatographically in dehulled grains during a storage period of 146 days in water at 3 C. Free ABA per grain decreased from 12.29 ng to 4.27 ng, and conjugated ABA from 7.14 to 4.86 ng during 146 days of storage.

The ABA contents of hulls, pericarps, embryos, and endosperms were determined in dormant and nondormant (120 days after storage) grains. Total ABA per gram dry weight of embryo and pericarp tissue exceeded that of hulls and endosperms by a factor of 50 in both dormant and nondormant grain. Total ABA per gram dry weight of embryos, pericarps, and hulls was significantly less in nondormant grain. The high concentration of ABA in dormant grain, especially in the embryo and pericarp, may be one cause of dormancy in wild rice grain.

Additional index words: Germination, Embryo, Pericarp, Endosperm, Hulls, ABA, Bound ABA, Dormancy, *Zizania palustris* L.

FRESHLY harvested grains of wild rice (*Zizania palustris* L.) require 3 to 6 months of moist storage at near freezing temperatures before germination will occur (Duvel, 1906; Muenscher, 1936; Simpson 1966). The physiological changes that occur during the cold, moist storage period which eventually release the seeds from dormancy have not been elucidated.

La Rue and Avery (1938) reported that embryos removed from freshly harvested grains would germinate on agar, but did not report on the vigor of these seedlings. Cardwell et al. (1973) and Woods and Gutek (1974) found that scraping the pericarp from above the embryo of dormant, freshly harvested grains with a scalpel after removing the lemma and palea resulted in 65 to 85% germination. Simpson (1966), using grains that had been held in cold storage for 96 days, reported that pricking the pericarp once above the embryo with a dissecting needle resulted in 92% germination compared to 4% germination for unpricked grains. Halstead and Vicario (1969) observed that treating dormant grain with ultrasonic vibration at 70 kg/s for 10 min resulted in 74% germination. They suggested that this was a result of pericarp disruption. Scarification by tumbling freshly harvested dehulled grain with granite in a small rock polisher for 80 to 100 min was 50% as effective in breaking dormancy as was removal of the pericarp with a scalpel (Oelke and Albrecht, 1978). Cardwell et al. (1978) found that pricking the pericarp above the embryo of freshly harvested grains would not induce germination, nor would removal of the pericarp from anywhere other than directly above the embryo. Apparently, the pericarp immediately above the embryo presents some type of barrier to germination.

The presence of germination inhibitors in wild rice grains has received little attention. Cardwell et al. (1978) reported that aqueous extracts of pericarps and hulls (lemmas and paleas) of freshly harvested grains decreased the germination of other scraped, freshly harvested grains by 77 and 84%, respectively. This discovery supports the hypothesis that inhibitors are located in wild rice caryopses and hulls.

At present, there is great interest in the hormonal control of seed dormancy and germination. The development of sensitive bioassays and analytical instrumentation have provided means to identify and quantify plant hormones.

Abscisic acid (ABA), a known growth inhibitor, has been identified in the dormant seeds of plum (*Prunus domestica*, cv. Italian) (Lin and Boe, 1972), peach (*Prunus persica* L. Batsch) (Diaz and Martin, 1972; Bonamy and Dennis, 1977), apple (*Malus domestica* Borkh.) (Balboa-Zavala and Dennis, 1977), and ash (*Fraxinus americana* L.) (Sondheimer et al., 1968). Although all of these seeds require a period of cold storage before germination and normal seedling growth, Bonamy and Dennis (1977) and Balboa-Zavala and Dennis (1977) reported that the decrease in ABA

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levels observed in peach and apple seeds was independent of temperature. They concluded that ABA alone was not responsible for dormancy in these seeds and speculated that cold storage is required for the synthesis of germination promoting substances. The suggestion has been made by Amen (1968), Khan (1971) and others that seed germination is controlled by a balance of promoters and inhibitors.

Khan (1971) reviewed the control of seed germination and dormancy by external application of hormones and suggested that this external control is a reflection of the natural control of dormancy and germination. Applications of ABA have inhibited the germination of seeds of several plants (Rudnicki, 1969; Sumner and Lyon, 1967; Sondheimer and Galson, 1966).

The objectives of this investigation were to determine if external applications of abscisic acid to wild rice grains inhibits germination and if abscisic acid is present in dormant wild rice grains and could be a factor in dormancy.

MATERIALS AND METHODS

Effect of Exogenous ABA on Germination

'K2' wild rice grains, harvested in September 1975, were submerged in water at 3 C and stored for 330 days. At that time 2,100 grains were dehulled and rinsed with water. After removal from cold storage, care was taken to keep the grains in ice water until they were used. The caryopses (dehulled grains) were blotted dry and scarified in a small rock polisher with crushed granite according to the method described by Oelke and Albrecht, 1978. After scarification, the caryopses were immediately submerged in prechilled solutions of ABA (mixed isomers obtained from Sigma Chemical Co.) for 16 hours at 3 C. Treatment concentrations were 0, 0.1, 0.2, 1.0, 2.0, 5.0, 10.0, and 20.0 mg ABA/liter. Four replications, each containing 75 caryopses for each treatment, were then put in an incubator for 16 hours at 23 C and 8 hours at 18 C. A photosynthetic photon flux of ca. 60 $\mu E\ m^{-2}\ sec^{-1}$ was supplied by a cool-white fluorescent lamp during the 16-hour period. After 7 days, germination counts were taken, the solutions poured off, and after rinsing twice with ca. 250 ml of water, non-germinated caryopses and the seedlings were placed in water for further germination and growth in the incubator. After 7 more days the water was again changed, germinated caryopses counted, and root and shoot measurements made. Final germination counts were made 21 days after the expermient was begun.

The term "germination," as used in this and succeeding experiments, is defined as coleoptile protrusion through the pericarp followed by coleoptile elongation. Coleoptile protrusion with no detectable elogation was not recorded as germination.

Endogenous ABA

Experiment I

Germination and Extraction. K2 wild rice grains were harvested on 30 Aug. 1976. Two days later an 800-grain subsample was removed and the remaining grains were stored in water at 3 C. Grains of the subsample were dehulled and fresh and dry weights of the caryopses determined. Four replications of 50 caryopses each were used to determine germination percentages and four replications of 100 caryopses each were used to determine levels of ABA. Germination tests were conducted in an incubator with a temperature and lighting regime as previously described.

ABA was determined as described for soybean tissue by Ciha et al. (1977). One hundred caryopses (fresh weight ca. 3.2 g) were homogenized (Polytron; PT-10-20; Brinkman Instruments) in 100 ml of 80% methanol at 4 C and extracted for 24 hours at 4 C. The homogenate was centrifuged at 1,545g for 15 min. The supernatant was collected and the sediment was resuspended in approximately 50 ml of 80% methanol and then centrifuged for an additional 15 min. The combined supernatants were split into two equal portions, taken to dryness in vacuo at 35 C, and stored at −20 C until analyzed. One portion was for determination of free ABA while the other was for total ABA (free ABA plus that released by hydrolysis). Samples used for total ABA determination were hydrolyzed in 5 ml of 0.1 *N* NH_4OH at 60 C for 1 hour. The samples were dried in vacuo at 35 C and stored at −20 C.

Preparative High Performance Liquid Chromatography (HPLC). The dried samples were reconstituted in 6 ml of 5% methanol in 0.2 *N* acetic acid and microfiltered through a 5-μm pore size teflon filter (LS series from Millipore Corp.). A known volume of the sample was subjected to preparative HPLC as described by Ciha et al. (1977). The eluate fraction containing ABA was collected, taken to dryness in vacuo at 35 C, and stored at −20 C until subsequent analysis.

Gas — Liquid Chromatography — Electron Capture (GLC-EC). The dry samples were reconstituted in 1.8 ml of 100% methanol to which 1.8 ml ethyl ether was added. The samples were then methylated (Schlenk and Gellerman, 1960) using diazomethane, dried under N_2, and reconstituted in 50 μliter pyridine to which 450 or 950 μliter of hexane were added. One μliter samples were injected into a gas chromatograph (Beckman GC-45) fitted with a 1.5-m × 2-mm i.d. glass column packed with 3% SP 2100 on 100/120 Supelcoport. Column temperature was 200 C and inlet and detector temperatures were 225 and 270 C, respectively. Methane at 5% (v/v) in argon was used as the carrier gas at 30 cm^3/min and as the carrier gas make-up at 100 cm^3/min added before the detector. A wide-range electron capture detector (Analog Technology Corp., Model 140A) with a scandium-tritide source was used. Levels of free and total ABA were determined directly. Conjugated ABA was calculated by subtracting the determined free ABA from total ABA.

Gas — Liquid Chromatography — Mass Spectrometry (GLC-MS). Methylated free ABA fractions were subjected to combined GLC-MS using an LKB-9000 Combined Gas-Liquid Chromatograph — Mass Spectrometer as previously described by Ciha et al. (1977).

Experiment II

Germination and Extraction. K2 grains used in this experiment were harvested on 28 Sept. 1977 from field-grown plants. Immediately, a 250-g subsample was removed and stored in water at room temperature. The remaining grains (ca. 750 g) were stored in water at 3 C. On 15 October the grains which had been held at room temperature were separated into hulls (lemma and palea), pericarps, and embryos and scraped off with a razor blade. The embryos with scutellums were peeled from the endosperms by hand. The different tissues were easily separated, therefore there was little cross contamination between tissues. Immediately after separation, the tissues were placed in 80% methanol as were samples of intact caryopses with hulls. All tissues were homogenized in 80% methanol and extracted for 24 hours at 4 C.

ABA determinations were made on four replications of 400 dissected grains and four replications of 200 intact grains. A fifth replication, 200 grains, was used for determination of tissue dry weights. Four replications of 100 seeds were used to estimate percent germination.

Purification and Analysis. Identical procedures as those described for Experiment I were followed for ABA purification and quantification, except that the GLC-EC was equipped with an automatic injector (Hewlett — Packard Model 7671A).

The entire procedure was repeated beginning on 28 Jan. 1978 with seeds from the same source that had been in cold storage for 120 days. At this time, the storage water was analyzed for the presence of free ABA.

Effect of Coating Wild Rice Caryopses with Petroleum Jelly

After hulls were removed dormant (freshly harvested) and nondormant (stored in water at 3 C for 90 days) caryopses were either scraped or left with pericarp intact. Half of the caryopses treated were coated with a layer of petroleum jelly before all caryopses were placed in beakers of water at 22 C to germinate. Germination counts were made after 20 days. Treatments contained 20 caryopses and were replicated twice. This experiment

is conducted to prevent leaching of ABA from the embryo ter pericarp removal.

RESULTS AND DISCUSSION

Effect of Exogenous ABA on Germination

All levels of exogenously applied ABA decreased ne germination percentage of nondormant wild rice aryopses (Fig. 1). Significant inhibitory effects on ermination were observed even at the lowest ABA oncentration of 0.1 mg/liter. No germination was btained at 10 or 20 mg/liter ABA. The amount of ABA which moved into the caryopses was not determined. Sumner and Lyon (1967) demonstrated that ABA, at concentrations similar to those used in this xperiment, inhibited the germination of grain of four rass species: veldtgrass (*Ehrharta calycina* J. E. mith); side oats grama (*Bouteloua curtipendula* Michx.) Torr.); annual ryegrass (*Lolium multiflorum* Lam.); tall fescue (*Festuca arundinacea* Schreb.). Transferring nongerminated veldtgrass grains to blotters soaked with water resulted in complete removal of inhibition of germination within 5 days. A similar reversal of ABA inhibitory effects on wild rice caryopses was not observed after rinsing and placing ungerminated caryopses in beakers of fresh water. Possible explanations of these results are that ABA causes irreversible changes to occur in the caryopses or that the ABA did not decline to a level below that which inhibits germination of wild rice grain.

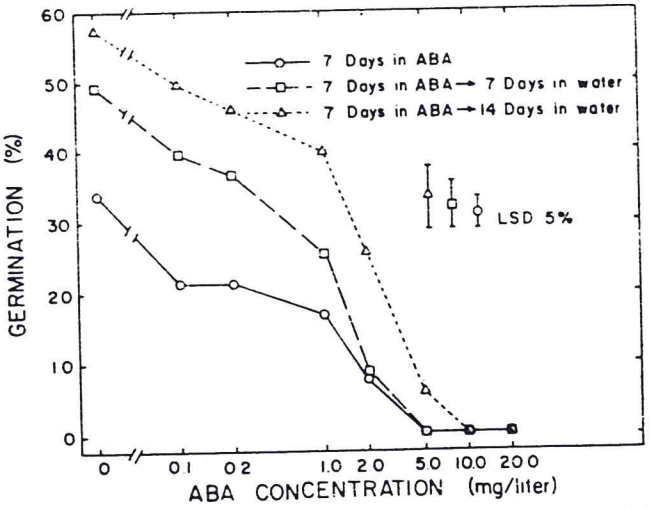

Fig. 1. The effect of exogenous ABA on germination of wild rice caryopses. A log scale was used for ABA concentrations.

Increasing ABA concentrations in pregermination treatments progressively reduced the percentage of seedlings which developed roots (Table 1). Similarly, increasing ABA concentrations caused a decrease in seedling shoot length. These observations suggest that ABA affects not only the germination triggering mechanism in wild rice caryopses, but also early seedling growth and development.

Table 1. Effect of exogenous applications of ABA during early germination on root and shoot development of wild rice seedlings.†

ABA‡ concentration	Plants with roots greater than 1 cm			Shoot length
	7 days in ABA	7 days in ABA + 7 days in water	7 days in ABA + 14 days in water	7 days in ABA + 7 days in water
mg/liter		%		cm
0	0	52.0	81.6	4.41
0.1	0	41.9	68.2	4.02
0.2	0	40.7	64.2	3.82
1.0	0	19.8	26.7	2.46
2.0	0	21.0	23.3	2.22
5.0	0	0	0	0
10.0	0	0	0	0
	L.S.D. 0.05	18.3	10.9	0.62

† Germination conditions: 16-hour day at 23 C and 8 hours at 18 C. Light was at 60 m E m^{-1} sec^{-1} from a cool-white fluorescent lamp.
‡ Nondormant wild rice caryopses were imbibed in ABA solutions at 3 C for 16 hours before being placed in the incubator in ABA for 7 days and later in water.
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Table 2. Caryopses weight, germination percent, and ABA levels as affected by storage in water at 3 C.

Days in storage	Wet wt.†	Dry wt.†	Germination	ABA levels					
				Total	Free	Conjugated	Total	Free	Conjugated
	g	g	%	ng/caryopses			ng/g dry wt.		
0	3.28	2.3	0 a*	19.70 a	12.29 a	7.14 ab	856 a	553 a	323 ab
16‡	3.19	2.1	0 a						
30	3.13	2.0	0 a	15.81 a	9.17 ab	5.30 b	759 a	481 a	273 ab
43‡	3.36	2.2	5.5 ab						
57	3.08	2.0	8.0 ab	14.56 ab	5.88 bc	8.68 a	705 ab	283 bc	422 a
70	3.09	2.0	15.0 b	14.25 ab	8.76 ab	5.55 b	699 ab	426 ab	269 ab
83‡	3.14	2.0	16.5 b						
96	3.14	2.1	15.5 b	9.29 b	4.29 c	5.03 b	441 bc	204 c	236 b
118‡	3.28	2.1	49.5 c						
131‡	3.27	2.1	54.0 c	9.14 b	4.27 c	4.86 b	420 c	197 c	223 b
146	3.31	2.1	71.5 d						
160‡	3.25	2.1	69.0 d						
174‡	3.25	2.1	73.5 d						

* Mean separation in columns by Duncan's multiple range test, 5% level of probability.
† Weight of 100 caryopses.
‡ ABA determinations were not made on these days.

Table 3. Effect of storage in water at 3 C on levels of ABA recovered from intact and dissected wild rice caryopses.

Tissue	Total ABA		Free ABA		Conjugated ABA	
	0 days†	120 days	0 days	120 days	0 days	120 days
	ng/tissue					
Embryo	33.94	20.80*	12.06	8.92	21.88	11.88*
Pericarp	24.71	18.55	9.49	7.66	15.23	10.91
Endosperm	11.69	9.14	4.57	4.20	7.10	4.93*
Hull	1.81	1.05*	1.15	0.62*	0.66	0.43
Total	72.15	49.55	27.27	21.40	44.87	28.15
Interact caryopsis with hull	59.90	42.66*	27.58	20.84	32.34	21.82*
	μg/g dry wt.					
Embryo	18.68	11.29*	6.65	4.85*	12.08	6.44*
Pericarp	13.43	10.28*	5.14	4.26	8.29	6.02
Endosperm	0.34	0.27	0.13	0.12	0.21	0.15*
Hull	0.28	0.13*	0.18	0.16	0.10	0.07
Total	1.62	1.15	0.62	0.50	1.01	0.65
Intact caryopsis with hull	1.34	0.93*	0.62	0.48	0.72	0.50*

* Pairs of means for 0 and 120 days for a particular tissue and form of ABA (total, free, or conjugated) significantly different at the 5% level of probability.
† Days in storage: caryopses with hulls intact were stored in water at 3 C. Germination at 0 and 120 days of storage was 0 and 52%, respectively.

Levels of Endogenous ABA

Identification of ABA. The methylated fraction of caryopses extracts subjected to GLC-EC showed a peak that corresponded to a methylated standard of ABA. The presence of ABA was further substantiated by subjecting several methylated caryopses extracts to GLC-MS. Fragmentation patterns were similar for both the methylated ABA standard and the caropses extracts. Major ions that appeared were 125, 134, 162, 190, and 260 m/e. The ratio of these fragments agreed with those observed by Bonamy and Dennis (1977) for ABA in fractions of peach embryo extracts.

Germination and ABA Content of Caryopses. There was a 54% decrease (19.70 ng/caryopsis to 9.14 ng/caryopsis) in recovered total ABA during 146 days of storage in water at 3 C (Table 2). Levels of recovered free ABA decreased by 65% (12.29 ng/caryopsis to 4.27 ng/caryopsis) and estimated levels of conjugated ABA decreased by 32%, (7.14 ng/caryopsis to 4.86 ng/caryopsis). What caused free and conjugated ABA levels to fluctuate on day 57 is not known, but could be due to inconsistencies in handling the grains after they were removed from cold storage. This could cause a biological conversion of free to conjugated forms. Loss of free ABA during purification and quantification of the extracts could also cause fluctuations in ABA levels. Since caryopsis weights were virtual-constant throughout the experiment, the data were nearly identical when examined on a per caryopsis or a per weight basis (Table 2). Germination percent increased with increasing lengths of time in cold storage (Table 2). Simpson (1966) and Halstead and Vicario (1969) previously reported that appreciable germination of wild rice grains did not occur until after 90 days of cold storage. In this experiment an increase in germination after day 43 could be due to disruption of the pericarp at the base of the caryopsis by removing the hull. The germination data for day 96 may be questioned since an incubator malfunction caused temperatures to reach 30 C during the germination test.

There appears to be a relationship between endogenous levels of ABA and germination capacity of wild rice caryopses. High levels of ABA and low germination percent are characteristic of freshly harvested caryposes. As length of time in cold storage increased, levels of ABA decreased and germination percent increased. A similar relationship was observed in 5 C stratified peach (Bonamy and Dennis, 1977) and apple (Balboa-Zavala and Dennis, 1977) seeds. These workers also reported a decrease in ABA levels in both peach and apple seeds that were stratified at 20 C, whereas germination percent increased only in seeds stratified at 5 C. It was concluded that chilling was not necessary for reduction of ABA levels in peach and apple seeds, but may be essential for the synthesis of germination promoting substances. Ash seeds also require stratification at 5 C before germination will occur (Sondheimer et al., 1968), but the seed's ability to metabolize ABA was the same whether stratification temperature was 5 or 25 C. (Sondheimer et al., 1974).

Cold temperatures may be essential for the synthesis of cytokinins (Borkowska and Rudnicki, 1975) and gibberellins (Sinska and Lewak, 1970) in apple seeds. The effects of warm storage temperatures on ABA levels in wild rice grains are not known nor are the temperature effects on gibberellin or cytokinin levels known.

Germination and Distribution of ABA in Wild Rice Grains. Intact, freshly harvested grains that were not subjected to cold storage did not germinate. Germination reached 52% after grains had been stored in water at 3 C for 120 days. Germination percentages were similar, approximately 84%, for freshly harvested and cold-stored caryopses if pericarps were removed from above the embryos. These results indicate that some grains were dormant after 120 days of cold storage. The germination percentage observed in this experiment are consistent with results of wild rice germination studies reported by Simpson (1966), Woods and Gutek (1974), and Cardwell et al. (1978).

ABA distribution in wild rice caryopses with hulls was approximately 47% in the embryo, 34% in the pericarp, and 19% in the endosperm and hulls (Table 3). During the cold storage period levels of all measured forms of ABA decreased, but not always statistically so in the embryo, pericarp, endosperm, hull,

e 4. Comparison of weight distribution of fresh and stored ld rice caryopsis.†

ıe	Days in storage	
	0	120
	g	
ıryos	0.73‡	0.73
carps	0.73	0.72
osperms	13.76	13.46
s	2.59	2.35
otal	17.81	17.26
ct caryopses with hulls	17.93	17.58

ıryopses with hulls intact were stored in water at 3 C.
y weight of 400 units of tissue.

d caryopsis with hull. Levels of conjugated ABA re higher than levels of recovered free ABA in all rts of freshly harvested and 120-day cold-stored yopses with hulls. Decreases in levels of conjugated BA transcended decreases in levels of free ABA in tissues except the hulls. A significant 46% decrease conjugated ABA in the embryo was the most dratic change observed. Levels of total ABA decreased gnificantly in the embryo hull, and intact caryopsis 39, 42, and 29%, respectively; while nonsignificant, tal ABA also decreased by 25% in the pericarp and % in the endosperm. It may be possible that ABA vels in caryopses and hulls would have decreased even lower levels after longer storage times since rmination was only 52% at 120 days.

Expressing levels of ABA on a per unit weight basis, ıe pericarp and embryo contain the highest concenations (Table 3). Because changes in dry weight of ıe tissues examined are negligable during 120 days of orage (Table 4), changes in ABA concentrations ıust be mostly due to changes in ABA amounts in the issue.

The recovery of high levels of ABA from the periarp and embryo may explain why removal of the periarp from immediately above the embryo promotes germination of freshly harvested caryopses that are otherwise dormant. Removal of the ABA containing pericarp and leaching of ABA from the exposed emryo area could cause a reduction of inhibitor content, thus permitting germination. Scraping also eliminates any mechanical barrier to germination that might be imposed by the pericarp.

Information supporting the hypothesis that an inhibitor is leached from the caryopsis after pericarp removal is presented in Table 5. Eighty percent of the scraped, dormant caryopses germinated, but coating the caryopses with petroleum jelly immediately after scraping reduced germination to 16%. Coating the nondormant caryopses with peroleum jelly did not affect germination. Either the petroleum jelly did not restrict water and gas movement into the caryopses or the inherent characteristics of wild rice prevented detrimental effects. The moisture content of the caryopses (freshly harvested) was about 30% at the beginning of the storage period and did not change during 174 days in water (Table 2). Thus, it appears water uptake is not essential for germination. Also, germination will occur under low oxygen tension. It is therefore quite possible that the petroleum jelly restricted the movement of some lipophillic substances (inhibitors) from the caryopses.

Table 5. Effect of petroleum jelly coating on germination of dormant and nondormant wild rice caryopses.†

Caryopses treatment	Germination	
	Dormant	Nondormant
	%	
Intact	0	71
Scraped	80	82
Intact + petroleum jelly	0	74
Scraped + petroleum jelly	16	78
L.S.D. 0.05	12	

† Dormant = freshly harvested seeds; nondormant = seeds stored in water at 3 C for 190 days.

Although release of ABA from the caryopses, either by removing some with the pericarp or leaching from the embryo, might be important in the breaking of dormancy by the scraping method, there is evidence suggesting that this is not the case in release from dormancy through cold, moist storage. The thick layer of wax on the external surface of the pericarp could be expected to hinder leaching of ABA from the grain. Analysis of 1.5 liters of water, in which approximately 1.5 kg of grains were stored for 4 months, resulted in the recovery of 4.6 μg of free ABA. It is estimated that the total loss of ABA from 1.5 kg of grains should be 330 μg. The discrepancy between observed and expected levels of ABA in the storage water could be a result of microbial degradation of ABA after it was released from the seed or a result of metabolism of ABA in the seed itself. Metabolism of ABA to phaseic acid and dihydrophaseic acid has been demonstrated in ash seeds (Sondheimer et al., 1974) and bean (*Phaseolus vulgaris*) shoots (Zeevaart and Milborrow, 1976).

Other evidence that metabolism might be more important than leaching of ABA in overcoming wild rice grain dormancy was supplied by Elliott (1975). Elliott reported 37 to 68% germination in grain stored at high humidity (not in water) at 3 C for 6 to 9 months. Grain handled in a like manner, but stored in water, germinated 70 to 100%. This information suggests that leaching conditions are not necessarily a prerequisite for the natural release of these grains from dormancy. If low levels of ABA must precede germination, then metabolism of ABA is suggested to be the main means to decrease ABA levels in wild rice grains.

Differences in absolute levels of ABA between grains harvested in 1976 (Table 2) and 1977 (Table 3) suggest that pre-harvest environmental factors may affect these levels. Since plant growth regulators control many plant functions in response to environmental stimuli, their levels in wild rice grains would be expected to vary from year to year. The findings of Goldbach and Michael (1976) that ABA levels in barley (*Hordeum vulgaris* L.) grains are sensitive to pre-harvest temperatures and water supply, support this hypothesis.

Although the magnitude of ABA decrease in wild rice grains was different for the two lots of grain examined, the overall decrease was significant in both years. If these decreases alone can account for the observed increase in germination is not known. An investigation of growth promoter levels through the cold storage period is warranted. The relationship of

growth inhibitors and promoters may be the critical factor in wild rice grain dormancy and not the absolute values of either.

Exogenously applied ABA had negative effects on germination of nondormant wild rice caryopses and subsequent seedling development. High levels of endogenous ABA, particularly in the pericarp and embryo, and low germination were characteristic of dormant, freshly harvested wild rice grains. After storage in water at 3 C, germination increased and levels of ABA decreased. Since exogenously applied ABA decreased the germination of nondormant caryopses, it is possible that the high levels of endogenous ABA in freshly harvested wild rice grains are at least partially responsible for their dormancy.

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