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GRAIN DORMANCY, PEROXIDASE ACTIVITY AND OXYGEN UPTAKE IN *ORYZA SATIVA*

EVELYN P. NAVASERO, LYDA C. BAUN and BIENVENIDO O. JULIANO

Chemistry Department, International Rice Research Institute, Los Baños, Laguna, Philippines

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Abstract—In developing grains of rice (*Oryza sativa* L.) of the dormant variety H4, peroxidase activity decreased sharply about a week before grain maturity without any change in grain dormancy and oxygen uptake of intact grain. During storage or after-ripening of mature dormant intact grains of four varieties (H4, H6, Mayang Ebos and Seraup 27) at 25–30°, the critical range in peroxidase activity was 1·0–1·4 μmol purpurogallin/hr/grain above which rice grains were almost completely dormant and below which the grains were almost completely nondormant. The oxygen uptake of intact H4 grain tended to decrease during the loss of dormancy. The decrease in both the peroxidase activity and oxygen uptake could be attributed mainly to the lower activities of the hull. Dehulling of developing and mature H4 grains reduced dormancy and increased the oxygen uptake of the grain. Thus, reduction by the hull of the level of oxygen available to the dehulled grain (embryo) was mainly responsible for grain dormancy in rice.

INTRODUCTION

Grain dormancy in rice (*Oryza sativa* L.) is not true embryo dormancy since excised embryos germinate readily [1,2]. The structures covering the rice embryo—the hull and the bran layer—probably play an important role in rice grain dormancy [3]. Dehulling has been shown to break dormancy partially and pricking the dehulled grain near the embryo breaks dormancy completely [1,4,5]. It has been suggested that the covering structures of the rice grain contain endogenous germination inhibitors [1,2] and that they create a barrier to the diffusion of oxygen in the embryo [4,6,7].

A comparison of dormant and after-ripened (stored 4–6 weeks at 25–27°) rough rice grains of the variety H6 indicated that during the period of soaking prior to visible germination, dormant and nondormant grains had similar levels of malate, total-soluble phenols and ethylene, and similar activities of leucine-[^{14}C] incorporation, maltase, and 3′-nucleotidase [5]. Only peroxidase activity decreased progressively during storage or breaking of dormancy [5,8]. Oxygen uptake data were inconclusive [5].

The present study investigated the changes in dormancy, oxygen uptake and peroxidase activity during seed development and storage of mature grain of a dormant rice variety H4 from Sri Lanka and the effect of dehulling on these properties. A Clark oxygen electrode was used for oxygen uptake measurements to verify whether or not peroxidase competes with the embryo for oxygen, thus, preventing the germination of dormant grains.

RESULTS

Intact grain. The intact grain of H4 rice air-dried for 2 days at 28–30° showed 100% dormancy (0% germination) during development up to maturity (28 days after flowering) and harvest (35 days) (Table 1). Peroxidase activity was constant during the first 3 weeks of grain development but started to decrease during grain desiccation in the field up to harvest. No significant trend in the oxygen uptake was observed.

Table 1. Peroxidase activity, percentage germination and O_2 uptake of developing H4 grains air-dried for 2 days at 28–30°*

Days after flowering	Intact grain			Dehulled grain†	
	Peroxidase activity (μmol purpurogallin/hr)	Germination (%)	Oxygen uptake (nmol O_2/hr)	Germination (%)	Oxygen uptake (nmol O_2/hr)
4	9·7	0	27·6	0	72·2
7	11·0	0	18·1	45	77·6
10	9·8	0	15·4	67	106·5
14	10·0	0	19·8	63	74·9
21	11·0	0	11·1		67·6
28	7·2	0	16·7		60·4
35 (harvest)	1·8	1	11·6	87	53·4
LSD (5%)	3·9	NS	NS	21	21·3

* Freshly harvested intact and dehulled grains failed to germinate.
† Hull included.

In the developing H4 grain the hull contributed most of the peroxidase activity. On a per grain basis, hull peroxidase activity was 56·2 μmol purpurogallin/hr in the 21-day grain, 67·4 μmol/hr in the 28-day grain and 11·4 μmol/hr in the 35-day grain. The dehulled grain (caryopsis or brown rice) had corresponding peroxidase activities of 8·7, 7·3 and 5·0 μmol purpurogallin/hr. The data indicated that the decrease in peroxidase activity of the rice grain during the week prior to harvest was mainly due to the decrease in activity of hull peroxidase. Baun [5] reported 75% of the peroxidase activity in the hull of a dormant mature sample in H4 rice grains.

Breaking dormancy in mature intact H4 grains by heat treatment (25–30° or 50°) was accompanied by a decrease in oxygen uptake of intact grain presoaked in H_2O for 2 hr and prior to germination (Table 2). The decrease in oxygen uptake was due mainly to a decrease of oxygen uptake by the hull fraction. By contrast, Chen and Varner [9] found the rates of oxygen uptake higher in nondormant than in dormant wild oats.

Storage or after-ripening of the mature dormant intact rice grains of H4 and three other varieties at 25–30° for 3–6 weeks resulted in the breaking of dormancy to less than 15% (85% germination) depending on the variety. Varietal differences in the after-ripening period required to break rice dormancy have been reported by other workers [4–8]. Peroxidase activity also decreased during storage (Fig. 1). Above the peroxidase activity of 1·4 μmol purpurogallin/hr/grain, all samples were almost completely dormant and below 1·0 μmol purpurogallin/hr/grain, all samples were almost completely nondormant. The peroxidase activity started to decrease during grain desiccation in the field (Table 1) and during early storage of the mature grain with no decrease in grain dormancy. The decrease in peroxidase activity in H4

Table 2. O_2 uptake and peroxidase activity of dormant, after-ripened and heat-treated H4 grains

Treatment	Germination of intact grain (%)	Oxygen uptake* (nmol/hr)				Peroxidase activity (μmol purpurogallin/hr)	
		Intact grain	Hull	Dehulled grain†	Embryo	Hull	Dehulled grain†
Dormant	13	20·9	13·4	24·8	11·5	1·07	0·60
After-ripened‡	83	14·6	7·1	29·3	11·0	0·57	0·58
Heat-treated§	89	13·6	8·9	23·0	10·7	0·58	0·51
LSD (5%)	9	5·0	4·2	NS	NS	0·11	NS

* Presoaked 2 hr prior to oxygen uptake measurement.
† Without hull. Dehulled grain was presoaked.
‡ 4 Weeks at 28–30°.
§ 2 Days at 50°.

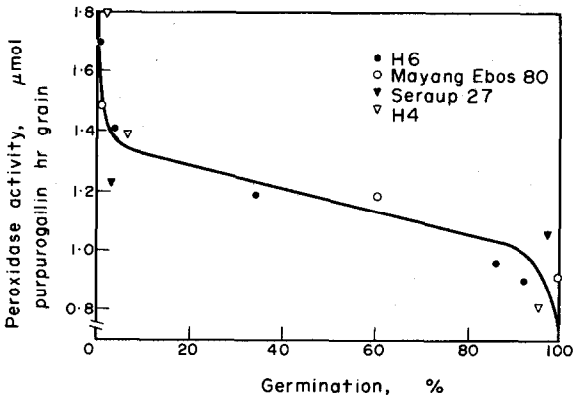

Fig. 1. Relationship between a decrease in dormancy, indexed by percentage germination, and the peroxidase activity during after-ripening at 25–30° of mature dormant grains of four rice varieties. H4 was stored at 28–30°, the others at 25–27°.

during after-ripening appears to be due mainly to the decrease in peroxidase activity of the hull (Table 2).

Zymograms of peroxidase extracts of dormant and nondormant H4 grains indicated the presence of five major isozyme bands in brown rice and two in the hull, using pyrogallol as substrate. The isozyme bands of the hull and of brown rice differed in their migration rates. Only the hull peroxidases decreased markedly in the intensity of the isozyme bands with loss of dormancy. With *o*-dianisidine as substrate, seven peroxidase bands were obtained for brown rice. Only the five faster migrating bands had lower intensities in nondormant samples.

Dehulled grain. Dehulling of the developing and mature H4 grains, air-dried for 2 days at 28–30°, resulted in a partial loss of dormancy except for the grains 4 days after flowering (Table 1). The rice embryo is not fully developed until 7 days after flowering [3]. Air-drying seemed to be required for germination since freshly harvested grain failed to germinate even after dehulling.

Oxygen uptake was consistently higher in the dehulled grain than for the intact grain (Table 1). Oxygen uptake by the dehulled grain was highest 10 days after flowering and decreased progressively with grain maturation. A similar trend in oxygen uptake was reported in the developing barley caryopsis [10]. Freshly harvested, dehulled grain, showed higher rates of oxygen uptake than the air-dried sample during the first 2 weeks after flowering. The decrease in dormancy to 13% (87% germination) caused by dehulling mature H4 grains was higher than the 40–80% dormancy (20–60% germination) reported for other varieties [4,5].

DISCUSSION

A decrease in grain dormancy (an increase in per cent germination) is accompanied by a decrease in the peroxidase activity (Fig. 1) and a decrease in the rate of oxygen uptake (Table 1 and 2) in mature H4 grains. However, there appears to be a critical value for oxygen uptake and peroxidase activity in dormant rice grains below which dormancy starts to decrease, and above which changes in oxygen uptake and peroxidase activity have no effect on dormancy, which remains at about 100% (0% germination). Varieties differ in the after-ripening period (25–30°) required to reach these critical levels. Air-drying of freshly harvested developing grains for 2 days at 28–30° seems to accelerate loss of dormancy. It is interesting that the decrease in peroxidase activity, as well as the decrease in oxygen uptake, during storage was due mainly to that of the hull, which is generally considered dead tissue. However, a contribution by the microbial population on the hull surface [11] to these properties cannot be completely eliminated since the peroxidase and oxygen uptake assays were not run under aseptic conditions.

The observation that dormancy of the mature rice grain can be broken by dehulling the grain suggests that the hull is a critical factor in dormancy. Since dehulling is accompanied by an increase in oxygen uptake in the dormant rice grain, the hull probably reduces the rate of oxygen diffusion into the caryopsis (particularly embryo). The oxygen uptake and peroxidase activity of the hull of dormant grain are higher than those of the hull of nondormant grain (Table 2). The other grain parts, dehulled grain and embryo, have similar peroxidase activities and rates of oxygen uptake in both dormant and nondormant samples. These data suggest also that availability of oxygen to the dehulled grain (particularly the embryo), as affected by oxygen uptake and peroxidase activity of the hull, is the critical factor controlling grain dormancy in rice. This interpretation is consistent with the breaking of dormancy of rice in a pure oxygen atmosphere [6].

Although the hull appears to be a major contributing factor in dormancy in H4 rice, the bran layer covering the embryo is probably as important as the hull in three other rice varieties previously tested [5]. The bran layer encloses the embryo in the rice grain [3]. Dehulling of these varieties reduced dormancy of the grain to only 80% in two varieties and 40% in another variety in contrast to 13% for H4. Pricking of dehulled grain of these varieties near the embryo also completely broke the dormancy as in H4 by allowing more rapid entry of oxygen to the embryo [5].

Dormancy does not appear to be due to a rudimentary or physiologically immature embryo since dehulled developing grain of H4 showed a high percentage of germination from 1 week onward after flowering (Table 1). Water-soluble inhibitors in the hull [1,2] were apparently unimportant in the varieties tested since the percentage of germination of grain was the same whether or not the hull was present in the germination medium for dehulled grain. Baun [5] reported little change in the level of total water-soluble phenols during after-ripening of dormant rice grain at 25–27°. Inhibitors in the embryo [12] are probably not involved since the percentage germination of dehulled grain pricked near the embryo was the same (100%) whether or not the pricked portion was in contact with the soaking water to allow such inhibitors to leach out [5].

EXPERIMENTAL

Dormant mature grains of H4, H6, Mayang Ebos 80 and Seraup 27 were harvested from the Institute's farm during the 1970 wet season. Samples of developing and mature H4 grains were obtained from the Institute's 1973 wet season crop from 4 days after flowering (anthesis) to maturity. Some samples were air-dried at 25–27° for 2 days and stored in airtight bottles inside a desiccator at 0–4° until used. Other samples were heated in an open container at 50° for 2 days or kept at 28–30° for 4 weeks to break dormancy [5,6]. A portion of the developing grains was freeze-dried for peroxidase assay.

Per cent germination was used as an index of dormancy, by the formula: % dormancy = 100−% germination. Germination tests were performed for 4 days at 28–30° in the dark on replicates of 50 or 100 grains soaked in H_2O for 24 hr according to Palmiano and Juliano [13]. Grains in which the radicle emerged within 4 days were classified as germinated. Dehulled grains were germinated in the presence of the hulls.

Oxygen uptake of 25 intact or dehulled grains and hulls and of 50 embryos was determined at 30° by a polarographic assay with a YSI oxygen monitor and a Clark oxygen electrode [14]. Grains or grain parts were presoaked for 2 hr in 5 ml H_2O containing 50 μg/ml each of nystatin (E. R. Squibb), tetracycline·HCl, streptomycin and penicillin [7]. Grains or grain parts were initially vacuum infiltrated for 3 min to remove air within the grain prior to assay for oxygen uptake. Four to five readings were made within 8–10 min.

Crude extract was obtained from 25 grains or grain parts soaked at 0–4° for 16–18 hr in 10 ml 0·1 M Pi buffer (pH 6·0), homogenized for 3 min and centrifuged at 30000 g for 10 min. This extract was assayed for peroxidase using pyrogallol as substrate [15] after Strength *et al.* [8]. Disc electrophoresis [16] was also performed on this extract and the peroxidase isozymes detected by incubation of the gel in 0·1% *o*-dianisidine or pyrogallol in acetate buffer in the presence of H_2O_2 [17].

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