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PROPHYLACTIC ROLE OF HULL PEROXIDASE IN THE DORMANCY MECHANISM OF RICE GRAIN[1]

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Abstract

It had been proposed that higher peroxidase activity of the hulls of dormant rice grains might deprive oxygen diffused through the covering structure, thus inhibited the germination of the seeds. This hypothesis was tested by determining the change of hull peroxidase activity during grain ripening, as well as by stratification treatment. It was found that when the grains ripened at lower ambient temperature, the grains were less dormant but their hull peroxidase activities were not lower than the grains ripened at higher temperature. Stratification treatment effectively terminated rice grain dormancy, but the hull peroxidase activity was hardly reduced. Thus the hull peroxidase hypothesis could not account for the grains which had experienced moist-low temperature conditions. In this context, a very sensitive response of respiration to temperature was found in the hulls compared with that of caryopses or intact grains. So under moist-low temperature conditions, the oxygen consumption of the whole grain is due to the seed within hull, and the embryo dormancy terminated independent of the hull peroxidase. Thus hull peroxidase prophylatically prevent rice grain from germination only when the caryopsis was still dormant.

Key words: Rice (*Oryza sativa* L.); seed dormancy; peroxidase.

Introduction

Yause (1970) detected peroxidase activity in mature rice grains and found that dormant grains possessed higher hull peroxidase activity than those of non-dormant grains. In addition to peroxidase activity, scientists of the International Rice Research Institute (1972) also found that the respiration rate of the hulls of dormant grains was higher. These observations may explain why dormant rice grains have higher oxygen uptake rate than non-dormant grains during the early phase of imbibition (Major and Roberts, 1968). Navasero *et al.* (1975) suggested that higher peroxidase activity in hulls might deprive oxygen that diffused through

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the covering structures, thus inhibited the germination of seeds. There were several evidences (Chu and Kuo, 1978) which supported the hull peroxidase hypothesis. But here we report some rice grains with both high hull peroxidase activity and high germinability.

Materials and Methods

Seasonal Effects

Two dormant rice (*Oryza sativa* L.) cultivars "Ta-Li-Ching-Yu" and "Karriranga" and one non-dormant cultivar "Taipei No. 306" were grown in the paddy for two seasons. The first crop was planted in the early spring (March), and ripened during hot summer (July, average temperature about 30°C). While the second crop met high ambient temperature during vegetative growth stage (August) and low temperature during grain ripening (November, average temperature about 20°C). Grains were collected from the developing ears of both crops of rice. Germination test was conducted at 30°C immediately after each collection, while the remaining samples were stored at −20°C until the determination of peroxidase activity.

Stratification Treatment

Grain lots of two dormant cultivars (San-Shih-Tzu and Ta-Li-Ching-Yu) were dry-stored in 4°C cool room. One hundred grains of each cultivar in three replications were weekly pooled and placed in Petri dishes containing one sheet of Whatman No. 1 filter paper and 10 ml distilled water. The Petri dishes were then stored in the same cool room prevented from drying out by plastic bags. After eight weeks all the stratified samples, together with the controls, were transferred to 30°C for germination test after washing with distilled water. For anaerobic stratification treatments, the same as above except that the grains were immersed under distilled water of 30 cm depth. Effects of stratification on hull peroxidase activity were determined with three dormant cultivars.

Enzyme Assay

Extracts for enzyme assays were prepared by grinding 20 detached hulls in 5 ml ice-cold 0.2 M phosphate buffer (pH 6.8) by a polytron (Kinematica). The extracts were filtered through Whatman No. 1 filter paper. Enzyme activity of the extract prepared by this method was not significantly different from the extract prepared by centrifuging at 4°C. Peroxidase activity was measured at 30°C by a modification of the method of Chance and Maehly (1955).

The reaction cuvette contained 0.75 ml pyrogallol (0.02M) and 0.025–0.2 ml extract. Phosphate buffer (0.02M, pH 6.8) was added to a volume of 2.55 ml. The reaction was initiated by the addition of 1 ml H_2O_2 (0.04M) to the cuvette. The initial velocity of the formation of purpurogallin, monitered spectrophotometrically at 430 nm, was calculated as the activity of peroxidase.

Measurement of Respiration

Grains of three cultivars as mentioned in "Seasonal Effects" were freshly collected seven days after anthesis. Samples were surface sterilized by 0.1% $HgCl_2$ for three minutes, then rinsed with distilled water. Some of the grains were carefully detached into hulls and caryopses. Ten intact grains, hulls or caryopses were placed in the manometric flask, together with 1 ml sterilized distilled water. Oxygen exchange rate was measured by standard method (Umbreit *et al.*, 1964) for the first 3 h at 30°C and 20°C for the following 3 h. The Q_{10} of the respiration was then calculated.

Results

The developing grains of the first crop were dormant up to the physiological maturity, irrespective of cultivars (Table 1). Grains of cultivars Ta-Li-Ching-Yu and Karriranga remained dormant 10 days after physiological maturity, while the germination percentage of cv. Taipei No. 306 increased rapidly 10 days after physiological maturity. Hull peroxidase activity reached a maximum before or at physiological maturity, and decreased thereafter. At full maturity (32 days after anthesis), the level of hull peroxidase activity of the three cultivars was in parallel with grain dormancy, well in agreement with hull peroxidase hypothesis. But at the late phase of grain ripening of the second crop, cv. Ta-Li-Ching-Yu, which remained dormant in the fist crop, showed a slight increase in germination, though the development of hull peroxidase activity was not significantly different from that of the first crop. Whereas the grains of cv. Taipei No. 306 had already reached a high germinability at physiological maturity, despite of the high activity of hull peroxidase at that time.

Figure 1 shows that dormancy of rice grains could be released by stratification treatment. The more dormant (i.e. slower rate of dormancy breaking under room temperature) cultivar San-Shih-Tzu was more resistant to the treatment than the less dormant cultivar Ta-Li-Ching-Yu. Longer duration of stratification decreased the viability of both cultivars, presumably by the infection of microorganisms. Oxygen might be a prerequisite to the effectiveness of stratification on rice grains, because deep immersion delayed the termination of dormancy. Again, the effect of anaerobiosis was more pronounced to the more dormant cultivar San-Shih-Tzu.

Although the dormancy of rice grains could be partially released by two weeks of stratification, the hull peroxidase activity was hardly affected (Table 2). The

Table 1. *Hull peroxidase activity and germinability of developing grains grown at different crop seasons*

The figures in the parentheses refer to the percentage of grains germinated. Both germinability and enzyme activity were tested at 30°C.

Days after anthesis	Peroxidase activity (ΔOD/min/hull)		
	Taipei No. 306	Ta-Li-Ching-Yu	Karriranga
	1st crop season (mean daily temp. 30°C)		
7	3.795 (0)	0.987 (0)	2.514 (0)
12	7.152 (0)	0.996 (0)	5.916 (0)
17	4.635 (0)	1.030 (0)*	3.702 (0)
22	3.487 (6)*	0.876 (0)	2.838 (0)*
27	2.052 (8)	0.492 (0)	1.608 (0)
32	0.284 (82)	0.372 (4)	1.221 (0)
LSD (5%)	0.288	0.058	0.094
	2nd crop season (mean daily temp. 20°C)		
12	7.998 (0)	0.945 (0)	2.727 (0)
19	9.336 (0)	0.863 (0)	4.632 (0)
26	10.494 (5)	0.810 (0)*	4.374 (0)
33	8.834 (72)*	0.615 (6)	3.138 (0)*
40	5.538 (98)	0.722 (26)	2.466 (2)
47	2.196 (98)	0.284 (42)	1.762 (6)
LSD (5%)	0.613	0.087	0.146

* Physiological maturity, that means the grains have reached maximum dry weight.

Table 2. *The effects of stratification on the germination and hull peroxidase activity of dormant rice grains*

Grains of the 3 cultivars were stratified at 4°C for 2 weeks, followed by germination test and determination of hull peroxidase activity, both at 30°C. Grains of controls were stored dry at 4°C during the period of treatment.

Cultivars	Treatmat	Germination (%)	Peroxidase activity (Δ OD/min/hull)
San-Shih-Tzu	Control	1.3	0.379 ± 0.082
	Stratified	46.7	0.366 ± 0.043
Ta-Li-Ching-Yu	Control	12.9	0.298 ± 0.046
	Stratified	72.0	0.310 ± 0.046
Karriranga	Control	2.3	1.023 ± 0.031
	Stratified	48.0	1.187 ± 0.293

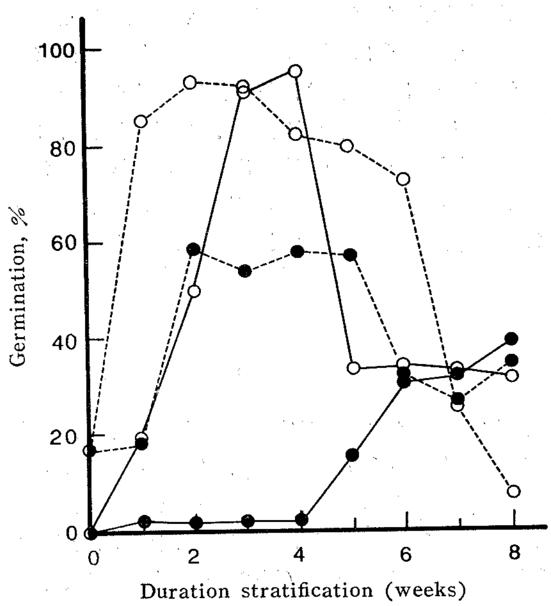

Fig. 1. Dormancy breaking effects of stratification under aerobic (○) and anaerobic (●, deep water immersion) conditions (cv. San-Shih-Tzu: ——; cv. Ta-Li-Ching-Yu: ------).

result clearly demonstrated that the inhibition of hull peroxidase to the germination of rice grains was not obligative.

The oxygen exchange rate of intact developing grains at 30°C was very small during the initial stage of imbibition, compared with that of the hull or dehulled grains (Table 3), suggesting an effective, although not complete prevention of oxygen into seeds by covering hull. This was supported by an unique high respiratory quotient of intact grains (data not shown). The Q_{10} (30–20°C) of the oxygen exchange rate of intact grains or caryopses were in the range of 1.0–2.1. But those of the hulls ranged from 8.0 to 15.3, indicating that the respiration of the hulls was very sensitive to temperature, although the possibility of high sensitivity of wound

Table 3. *Oxygen exchange rate ($\mu lO_2/h$) of intact rice grains, hull and caryopses imbibed at 30 and 20°C*

	Taipei No. 306			Ta-Li-Ching-Yu			Karriranga		
	Rate		Q_{10}	Rate		Q_{10}	Rate		Q_{10}
	30°C	20°C		30°C	20°C		30°C	20°C	
Grain	2.42	1.59	1.5	1.99	1.22	1.6	1.59	1.56	1.0
Hull	7.93	0.52	15.3	6.48	0.74	8.8	11.00	1.38	8.0
Caryopsis	9.47	4.45	2.1	10.37	5.86	1.8	10.89	5.52	2.0

respiration of the detacted hull to temperature was not excluded. While the Q_{10} (20–10°C) of the hull was around 2, the same magnitude as those of whole grains or dehulled caryopses (data not shown). Varietal differences also existed in the Q_{10} of the respiration of the hulls, with cv. Taipei No. 306, the non-dormant one, ranked the most sensitive among the three cultivars tested. From Table 3, we suggest that at 30°C, the oxygen consumption of the whole grain is largely due to hull, but at 20°C, the oxygen consumption of the whole grain is largely due to the seed within hull.

Discussion

In many species, environmental conditions which are experienced by developing seeds can affect the formation of dormancy. The results of most investigators indicate that more dormant seeds may be formed under longer daylength, while the effects of temperature are less consistent (Kuo, 1980). Lin (1955) reported that rice grains harvested in the first crop (high ambient temperature) were more dormant than those in the second crop (low ambient temperature). Ikehashi (1973) claimed that higher temperature during later period of grain-filling produced more dormant rice grains. But the results of Hayashi and Hidaka (1979) were just the contrary. Our results are consistent with the former. So far we can afford no explanation to these disagreements.

In addition to 70 species of Gramineae seeds which are sensitive to low temperature imbibition (Roberts and Smith, 1977), we find that the grain dormancy of rice, which is of tropical origin, can also be effectively terminated by 2 to 4 weeks of stratification. The stratified grains germinated readily under ambient temperature of 30°C, although their hull peroxidase activities, which were found (Navasero *et al.*, 1975; Yasue, 1970) to be correlated with the germinability of the grains, were hardly reduced by the treatment. The results suggest that the embryo of stratified rice grains do not require oxygen for germination. Nevertheless, oxygen still plays an essential role in dormancy breaking effects of stratification, because anaerobiosis delays the termination of dormancy (Fig. 1).

Côme and Tissaoui (1973) proposed that phenolic compounds which were rich in seed coat tissue might fix oxygen dissolved in seed coat and impaired the germination of apple seeds. This mechanism of dormancy may not be applied to the cereal grains, due to firstly, the activity of polyphenol oxidase in the covering structure of barley grains does not decrease with after-ripening (Roberts and Smith, 1977), and secondly, both polyphenol (Navasero *et al.*, 1975) and diphenol contents (Chu and Kuo, 1978) are not correlated with the degree of dormancy of the grains among different cultivars, or between seeds lots of the same cultivars.

We propose that the embryos receive oxygen more readily when the grains stratified. This may be realized by higher solubility of oxygen at low temperature or failure of oxygen consumption of hull at low temperature. The high sensitivity of respiration of detached hull to temperature (Table 3) supports the latter possibility. The grains with intact hulls have Q_{10} of respiration a little smaller than those of the caryopses, far less than those of the detached hulls. It is reasonable to suggest that the oxygen consumption of the intact grains is contributed largely by the hulls at temperature of 30°C. But at 20°C the caryopses take a larger part. The explanation may be applied to both the stratified grains and the ripening grains of the second crop.

Kono *et al.* (1975) found that dehulled dormant rice grains failed to germinate when immersed in deep water, but did germinate in Petri dish with adequate oxygen supply. While the germination of non-dormant grains was not affected by anaerobiosis. The results indicate that once the dormant stage has been terminated, the rice grains require oxygen no more than for radicle protrusion. Thus the role of hull peroxidase to grain dormancy of rice is only prophylactic, i.e., it is effective on causing grain dormancy only when the embryo is relatively dormant. When dormant grains imbibed, the hulls may consume most of the oxygen diffused through the covering structures, and prevent the grains from germination. But during stratification or ripening under low ambient temperature, the respiration rate of the hulls will be temporarily reduced, and the hulls do not effectively prevent oxygen from reaching embryo. Thus the oxidative process vital to germination may have been proceeded in the embryos at the end of stratification or ripening period. When these grains are imbibed at room temperature, they will germinate in spite of high oxygen consumption of the hulls.

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稻殼過氧化酶：水稻穀粒之預防性休眠機制

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有學者倡議休眠水稻穀粒之稻殼過氧化酶活性較高，將透過稻殼的氧消耗，導致種子無法發芽。本研究就種子充實過程及層積（低溫浸潤）處理對稻殼過氧化酶活性的影響來檢討稻殼過氧化酶假說。在臺北第二期作低氣溫環境下成熟的穀粒，其休眠性較第一期作高溫時期充實者爲低，但是稻殼過氧化酶的活性並不比第一期作者低。層積處理有效地解除水稻穀粒休眠，但對稻殼過氧化酶活性沒有影響。因此該假說無法說明經驗過低溫濕潤時期的穀粒的低休眠性。由於低溫降低稻殼呼吸作用的幅度遠大於穎果或完整穀粒，因此在低溫濕潤時，水稻穀粒之氧氣消耗主要的部位是種子而非外殼。此期間胚得到足够的氧而解除休眠。所以在發芽試驗時稻殼過氧化酶的活性雖高，但已不足以阻止種子發芽。因之稻殼過氧化酶的抑制水稻穀粒發芽是預防性的，只在穀粒解除休眠之前有效。