Inhibitory Effects of Oxygen on Seed Germination as a Specific Trait of *Japonica* Rice, *Oryza sativa* L.

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Dormancy in cereal seeds of oat, barley and rice is usually broken by a higher oxygen tension. In this experiment, however, the inhibitory effect of high oxygen tension on seed germination was observed in the seeds of *japonica* rice, unlike in those of *indica* and wild type rices. Standard dehusking, which increases the supply of oxygen to the embryo of a seed and the increment of the oxygen tension in the atmosphere surrounding an intact seed strongly inhibited germination of rice for a certain period of time after harvest. This inhibition was not observed under anaerobic conditions, such as in water and at a low oxygen tension. The inhibitory effect was especially recognizable in *japonica* rice seeds with a high water content irrespective of whether they were dormant or non-dormant cultivars, but not in *indica* or in wild rice (*O. perennis* MOENCH and *O. sativa* f. *spontanea* ROSCHEY).

KEY WORDS : *Oryza sativa* L., *indica*, *japonica*, oxygen inhibition, seed germination, aquatic plants.

Introduction

It is difficult for the embryo enclosed within the seed integuments or the fruit wall and sometimes both, to be in contact with oxygen in the surrounding atmosphere unlike the growing plant with an aeration system. Intact seeds in many species require higher oxygen tension for germination than excised embryos. Oxygen availability for seed germination is linked with the breaking of dormancy in many cases. Dormant cereal seeds including wild oat, barley and rice are compelled to germinate at a higher oxygen tension (CROCKER and BARTON 1953). However, there are other species, including a number of aquatic plants in which germination is promoted under low oxygen tension, for example *Cynodon dactylon* Persoon, *Poa compressa* L., *Typha latifolia* L. (MORINAGA 1926 a, b) and *Zizania aquatica* L.

The breaking of dormancy of rice seed is enhanced by a higher oxygen tension (ROBERTS 1961). However, TAKAHASHI and OKA (1957) reported that the seed dormancy of rice can be broken by anaerobic incubation. To clarify this discrepancy, one of the authors examined the inhibitory effect of oxygen on seed germination in *japonica* rice (TAKAHASHI 1985). The present paper describes in more detail the inhibitory effect of high oxygen tension on seed germination in *japonica* rice, which was not observed in *indica* and wild rice.

Materials and Methods

Materials :

The seeds of fourteen rice cultivars and two wild rice plants were used in this study (Table 1). Eleven rice cultivars belonged to the *japonica* group and three to *indica*. *O. perennis* Moench (perennial type) and *O. sativa* f. *spontanea* Roschey (annual type) were

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used as wild rice. These rice cultivars were grown in the experimental fields of the Institute for Agricultural Research, Tohoku University. Three photoperiod sensitive cultivars and two wild rice plants were grown in Wagner pot (1/5,000 a, 15 cm diameter pot) and their flower buds were artificially induced by short day treatment (8 hr photoperiod for 2 weeks) at the tillering stage. Ear emergence from the flag leaf sheath occurred in all the plants in August. Grains from these plants were harvested at the maturity stage when the covering layers of seed (husk and seed coat) lost their chlorophyll and seed dry matter reached maximum values.

Table 1. Rice cultivars and wild rice plants used

<i>Japonica</i> rice	<i>Indica</i> and wild rice
Kamenoo	Surjamukhi
Toyokuni	Peta
Shirakawa	Shirokara
Toogo	<i>Oryza sativa</i> f. <i>spontanea</i> ROSCHEY
Aikoku	<i>O. perennis</i> MOENCH
Rikuu no. 132	
Fujisaka no. 5	
Fujiminori	
Fukubouzu	
Sasaminori	
Sasanishiki	

Methods :

(1) Seed germination

Germination tests were carried out on the intact and the dehusked seeds, both at the time of harvest and after dry storage. Freshly harvested seeds were dried at 40°C for 3 days in an air circular system and then they were stored in a desiccator with silica gel for one month to become the dry seeds used in this study. The water contents of the freshly harvested and dry seeds were determined by drying at 80°C for 3 days in an air circular system. The moisture contents of the fresh and the dry seeds per dry matter were 22 to 39 per cent and 11 to 14 per cent, respectively.

Seeds were sterilized with 70 per cent of ethanol solution for 5 min. and after washing, sterilized again with 2.0 per cent of sodium hypochlorite solution for 10 min. and then rinsed with sterilized water three times. Husk was carefully removed from the intact seeds with a forceps under a magnifier.

Seeds in each set were placed either on a moistened filter paper in a Petri-dish 9 cm in diameter (Experiment 1) or on a moistened filter paper in a glass vessel for germination (6 cm diameter × 10 cm high), so as to maintain a 50, 20 or 0 per cent oxygen tension in nitrogen gas (Experiment 2), or in 5 cm depth of water in the germination vessel mentioned above.

The number of seeds from which the radicle or plumule had emerged through the husk or seed coat was recorded at 24 hr intervals during the 10 day incubation period at 30°C in the dark, using two replicates of 50 seeds for each treatment.

(2) Experiment 1 (Effect of dehusking on germination under aerobic conditions)

The effect of dehusking on the germination of freshly harvested seeds was determined by comparing the numbers of intact and dehusked seed that germinated. The dehusking effect was also examined by using the dry seeds as mentioned above.

(3) Experiment 2 (Effect of dehusking on germination under anaerobic conditions)

The germination of freshly harvested seeds in *japonica* rices was measured by incubation of intact and dehusked seeds either on two moistened filter papers, or during immer-

sion in water or under different oxygen tensions, as mentioned already. The effect of low oxygen tension (anaerobic effect) was evaluated comparing the number of intact and dehusked seeds which germinated in water and at different oxygen tensions. From the beginning of this experiment, each mixed gas was flowed into each vessel by velocity at the rate of ten liters per day until the completion of the experiment.

Results

Table 2 shows the effects of dehusking on seed germination of *japonica*, *indica* and wild rice. The intact seeds of some of the *japonica* cultivars had lost their dormancy and were able to germinate completely at harvest time, whereas when the seeds were dehusked, germination was strongly inhibited. The inhibition was also recognized in the dormant seeds, such as Fukubouzu and Aikoku etc. (Table 2).

The inhibitory effect of dehusking on germination was conspicuous in the seeds which had high water contents (above 30 per cent in Table 2). In *indica* rice seeds, however, freshly harvested seeds showed a strong dormancy and the dehusking of the seeds did not inhibit the germination, but instead promoted it. The seeds of these cultivars also had relatively high initial water contents although the moisture contents were somewhat lower than in the *japonica* rice seeds (Table 2). The results obtained in *indica* rice seeds were comparable with the data of ROBERTS (1961). Table 3 shows that the inhibition induced by dehusking was removed in dry seeds.

Fig. 1 indicates that the inhibitory effect of dehusking on germination of freshly har-

Table 2. Inhibitory effect of dehusking on germination of freshly harvested seeds

Cultivar	Intact seeds		Dehusked seeds		Water content of intact seeds 1978
	1976	1978	1976	1978	
	%	%	%	%	%
<i>Japonica</i>					
non-dormant cv.					
Kamenoo	98	100	11	16	37.9 ¹⁾
Toyokuni	100	100	15	12	38.8
Shirakawa	90	95	10	8	32.1
Fujisaka no. 5	100	100	29	13	34.9
Sasanishiki	100	98	57	26	33.7
dormant or partially dormant cv.					
Toogo	—	15	—	5	40.6
Aikoku	—	25	—	4	43.4
Fukubouzu	—	60	—	7	42.3
Rikuu no. 132	—	75	—	16	35.8
Fujiminori	—	78	—	14	32.8
<i>Indica</i>					
Surjamukhi	—	15	—	35	24.5
Peta	—	25	—	65	21.8
Shirokara	—	19	—	30	29.0
Wild rice					
<i>O. sativa f. spontanea</i>	—	0	—	0	19.1
<i>O. perennis</i>	—	0	—	0	17.8

¹⁾ Numeral shows water content per dry matter of seed.

vested seeds was not recognized when the dehusked seeds were immersed into water. The results obtained in Fujisaka no.5 seeds coincided with the author's preliminary report using cultivar Sasanishiki (TAKAHASHI 1985). Moreover, the results in Table 4 show that the higher oxygen tension remarkably depressed the germination of dehusked seeds, whilst the absence of oxygen removed the inhibition of seed germination in *japonica* rice. In contrast, dehusking and exposure to a higher oxygen tension promoted the germination of *indica* seeds (Table 4).

These data indicate that for a certain period of time after harvest, anaerobic conditions are favorable for the germination of freshly harvest seeds in *japonica* rice, whereas this phenomenon could not be observed in dormant and non-dormant seeds of *indica* rice. In *indica* rice dehusking tended to somewhat promote rather than inhibit germination, irrespective of the degree of dormancy. The results obtained in wild rice seeds were similar to those obtained in *indica* seeds (Table 3).

Fig.1. Effects of dehusking on seed germination under aerobic (on filter paper) and anaerobic (in water) condition.

Table 3. Effect of dehusking on germination of stored dry seeds¹⁾

Cultivar	Intact seeds	Dehusked seeds	Water content of intact seeds
	%	%	%
<i>Japonica</i>			
Kamenoo	100 ³⁾	100 ³⁾	11.5 ²⁾
Toyokuni	100	100	12.0
Sasanishiki	100	100	11.3
<i>Indica</i>			
Surjamukhi	36	100	12.1
Peta	75	98	12.0
Shirokara	80	100	13.5
Wild rice			
<i>O. sativa f. spontanea</i>	35	72	11.5
<i>O. perennis</i>	22	49	12.0

¹⁾ Seeds were dried at 45°C under air flow for 2 weeks and then were stored in a desiccator with silica gel for one month.

²⁾ Numeral shows water content per dry matter.

³⁾ Percentage of germination is shown.

Table 4. Effects of oxygen tension on dehusked seed germination in *japonica* and *indica* rice

Cultivar	Germination percentage								
	0% O ₂			20% O ₂			50% O ₂		
	I.	D.	(diff)	I.	D.	(diff)	I.	D.	(diff)
<i>Japonica</i>									
Fujisaka no. 5	100	100	(0)	98	11	(-87)	65	4	(-61)
Sasanishiki	100	100	(0)	100	25	(-75)	75	3	(-72)
Kamenoo	98	100	(+2)	100	31	(-69)	87	13	(-74)
<i>Indica</i>									
Surjamukhi	0	0	(0)	18	41	(+23)	29	52	(+23)
Peta	0	0	(0)	23	93	(+40)	35	71	(+36)
Shirokara	0	0	(0)	21	54	(+33)	45	68	(+23)

I : intact seeds, D : dehusked seeds, Parenthesis shows difference between germination percent of I and D.

Discussion

Covering layers or seed coats may be sufficiently impermeable to oxygen to prevent seed germination from occurring in many plants. Therefore, intact seeds of these plants require a higher oxygen tension for germination. It is generally recognized that in the dormant cereal seeds, especially those of oat and barley, germination is promoted by a high oxygen tension. The breaking of seed dormancy in rice is also enhanced generally by a higher oxygen tension (ROBERTS 1961).

In this study, however, the inhibitory effect of oxygen of seed germination was confirmed in *japonica* rice whose seeds were freshly harvested and had maintained high water contents (above 30 per cent herein). This inhibition occurred only for a certain period of time after harvest and then disappeared after dry storage of seeds.

The phenomenon identified in *japonica* rice seeds is probably related to the fact that the inside of freshly harvested seeds is likely to be subjected to anaerobic conditions due to the presence of the covering layers. In general, the seeds of aquatic plants tend to germinate under a low level of oxygen (MORINAGA 1926 a, b). Germination ability of *japonica* rice seeds under anaerobic conditions seems to be one of the characteristics of aquatic plants. Seeds of wild aquatic plants in the temperate region, such as *Zizania aquatica* L. seeds, can not germinate immediately after maturation because of the development of dormancy and of unfavorable cool temperature of germination. In the seeds located in moistened soil dormancy persists throughout the winter. Seeds have to pass through an after-ripening period under freezing or near-freezing temperatures before dormancy can be broken. And then in the next spring seeds germinate under anaerobic conditions, such as in the bottom of a river and lake (SIMPSON 1966, DORE 1969).

After ageing or drying, *japonica* rice seeds did not show the effect of oxygen on germination as in the case of terrestrial plant seeds. The viability of *Zizania aquatica* L. seed is lost by drying (DORE 1969). It thus seems that the seeds of *japonica* rice show both aquatic (freshly harvested seeds) and terrestrial (dehydrated seeds) traits.

On the other hand, in *indica* rice dehushing promotes rather than inhibits germina-

tion, while the seeds usually show a strong dormancy as compared with *japonica* rice seeds. The behavior of wild rice seeds was similar to that of *indica* seeds.

The elucidation of the inhibitory mechanism of oxygen on seed germination in rice as an aquatic plant requires for the studies along with the analysis of the basic difference between oxygen effects on seed germination in *indica* and *japonica* rice.

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日本イネの特異性としての発芽におよぼす酸素の阻害効果

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普通, 種子の胚は, 包被組織(種皮・果皮ときには果肉)によって包まれており, ほかの植物組織のように通気系をもっていないため, 空気中の酸素と接触することが困難である。

このため多くの種子は, 発芽するときに気中よりも, より高い酸素分圧を要求することが多い。とくに休眠状態にある種子では, 高酸素分圧による休眠打破の効果が認められている。たとえば野生のエンバク, オオムギあるいはイネなどをはじめイネ科植物の休眠種子は, 高い酸素分圧の下で発芽が誘導されることが知られている。

一方, 多くの水生植物では, 逆に低酸素分圧で発芽が促進され, 種によっては酸素分圧を高めることによって, 発芽力を失なうことも報告されている。一年生のマコモ(所謂アメリカ野生稻, *Zizania aquatica* L.)などはその代表とされている。

イネの休眠種子は, 一般に酸素分圧を高めることによって発芽が誘導されるものと考えられてきた。E. H. Roberts (1961) は, 一連の実験を行なうことによってこの事実を強調した。しかし, 著者ら(1957) は, 酸素欠乏下でイネの休眠が打破されうることを実証し, Roberts の説との間に矛盾が存在していることを指摘してきた。このことに関連し, TAKAHASHI (1985) は, 日本イネの種子について, 酸素分圧を高めることによって, 発芽が阻害される事実のあることを予報した。本報はこれらの不明瞭な問題点を明らかにするため, 前報にひきつづきより詳細な実験を試みた結果を取り纏めたものである。

インディカ, ジャポニカおよび野生イネを用い, 収穫直後の種子および乾燥貯蔵した種子について検討した。用いた品種はいずれも南東北地域で充分種子が成熟しうるように, 必要に応じて短日処理をほどこして出穂を調節し, 正常な種子を得るように努めた。

本研究の結果はつぎのようである。日本イネの種子は, 収穫時に既に休眠を消失し完全に発芽可能であるか, または部分休眠の状態にある品種が多い。このような種子では収穫直後の一定期間, 酸素供給を高める処理, たとえば, 除穎によって種子のまわりの酸素分圧を高めると, 発芽が強く抑制される。

この現象は, 部分休眠の程度には関係なく認められる。しかしこのような状態にある種子を除穎して, 水中または酸素分圧の低い条件下におくと発芽阻害が認められない。収穫直後の種子は, 水分含量が一般に高く,

種子の乾物当り 30% 以上の含水率を保っているものが多い。収穫直後の種子胚は、包被組織に包まれて水分含量が高く嫌氣的条件下にあるものと推定され、かかる条件下で日本イネの胚は生長しうる生理的条件を保持しているものと推定される。

種子を乾燥し貯蔵しておく、水分低下とともに上述した高酸素分圧による抑制現象は現われず、一般に従来認められているように除根や高酸素分圧による発芽促進が確認されるようになる。

日本イネに認められる、かかる高酸素分圧による発芽阻害現象は、インド型イネや野生イネでは認められず、収穫直後の休眠状態にある種子を始め、休眠消失過程にある種子でも、いずれも除根や酸素分圧の高まりによって発芽が促進される。

以上、本報では生態を異にするイネ亜種（生態種）であるインディカとジャポニカに認められる上記特性について若干の議論を行った。