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Evaluation of the Seed Germination Vigor of Rice Varieties by Sodium Dithionite Treatment

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Poor seed germination and uneven early growth are often observed in rice varieties when the seeds have been directly sown into the flooded paddy field. The uniform germination followed by the vigorous early growth is regarded as an essential character for the rice genotypes having a high and stable productivity in the direct sowing cultivation system. In this research we examined the characteristics in germination and early growth of 17 varieties of 0. *sativa* and 0. *glaberrima* under the deoxidized water condition. Oxygen in water was removed by sodium dithionite. The germination ratio of 0. *glaberrima* was strongly restricted in the deoxidized water, while the restriction on *0. sativa* was not so strict. There was little difference in germination ratio among the varieties of 0. *sativa* tested in the deoxidized water, but a large difference was detected for the early growth after germination. In the deoxidized water, the two varieties (Razza77 and Calrose76) among the **15** varieties of 0. *sativa* had a continuous growth, while the other 13 varieties had no response in growth after germination. It may be expected that these two varieties have a high enzymatic activity in the anaerobic respiration metabolism to get energy necessary for the initial growth. The responses of germination and early growth of rice varieties to the deoxidization treatment had a good agreement with those determined in the flooded paddy soil. This may prove that the germination test in the deoxidized water is useful for screening the rice genotype suitable to the direct sowing cultivation.

INTRODUCTION

The germination is defined as a physiological and biochemical process composed of many complicated responses which take place in a seed at the stage from the beginning of water absorption to the appearance of radicle or plumule. Water, adequate temperature and oxygen are required as the essential elements to synthesize the energy for initiating the germination and growth, the process of which has been summarized by Ching (1982). Also a light illumination to seed is an additional element for germination in some species. The germination and early growth are important determinants for crop production, and the vigorous and uniform germination is prerequisite to the stable and high production.

On the germination of rice species and varieties, the detailed studies have been conducted in series in both physiological and genetical fields (Takahashi, 1953, 1962, 1985; Takahashi et al., 1976). Water and adequate temperature are the indispensable elements for germination of rice. It has been reported that many of rice varieties are able to initiate germination at low O_2 concentrations because the species have a relatively high capacity of producing energy by the anaerobic respiration.

In Japan the transplanting cultivation system has been widely used for rice production. In this system the seedlings are grown in the nursery boxes with adequately

controlled environments, where the oxygen concentration in the soil is not a restricting factor for the germination and early growth. Recently the direct seed sowing in the flooded paddy field has been re-examined to save labor energy in the rice cultivation. In this system the poor germination and uneven early growth have frequently occurred depending on oxygen deficits in the soil. These are regarded as a considerable cause of the low and unstable production. Although rice varieties usually present a relatively high vigor in germination with a high tolerance against low O_2 concentration, yet the germination seems to be often restricted under the extremely deoxidized condition in the soil of flooded paddy.

To promote the germination in the paddy soil, the oxidizer -covered seeds have been used (Ota and Nakayama, 1970; Hagiwara ***et al.,*** 1987). In order to get a high yield in the direct sowing cultivation system, it is fundamentally important to use the genotypes with a high activity in germination and early growth under the oxygen deficit conditions. In this experiment, using 17 cultivars of *O. sativa* and *O. glaberrima* from the different countries, the germination tests were conducted in the deoxidized water, and the characteristics of germination and early growth were investigated and compared among the varieties.

MATERIALS AND METHOD

Experiment 1. Investigation of germination and early growth under the aerobic and anaerobic conditions.

Materials

The seeds of 17 varieties (15 varieties of 0. *sativa* and 2 varieties of 0. *glaberrima*) harvested in the experimental paddy field of Kyushu University in 1989 were stored at 5 °C in the refrigerator until the germination test was conducted in August to September in 1990.

Observation of the germination in water

Germination was recorded as the number of seeds the radicle or plumule of which had emerged through the hull. Sixteen seeds for each variety were fixed in the small holes which were cut open at regular intervals on a round cotton flannel (6 mm in thickness and 90 mm in diameter). This set was placed on the bottom of a beaker (500 ml) filled with distilled water and incubated for 5 days at 30°C in the dark. The mouth of the beaker was uncovered so that a certain level of oxygen concentration could be maintained in the water. From the number of germinated seeds under this condition (the control plot), the germination ratio was calculated by the equation shown below.

Germination ratio (%) = (The number of seeds germinated by the observation day) / (The total number of seeds tested)

The seeds of 0. *glaberrima* failed to germinate because water could not penetrate their hulls. The seeds of this species were hulled in advance and used for the germination test.

The deoxidized water plot was provided; the results obtained here were compared to those of the control plot. Anaerobic condition was created by adding O.lg of sodium dithionite ($Na_2S_2O_4$) into the distilled water in a 500 ml beaker, and the mouth of the beaker was covered with a parafilm to prevent air dissolution into the water. On

calculation, O.lg of $Na_2S_2O_4$ can absorb 14.3ml of oxygen at 30°C. The soluble oxygen volume in 500ml water is about 2.6ml at 30°C; hence, by this treatment the seeds in the water were placed under the completely O_2 -free condition. The tests were conducted with 5 replications for each variety.

The germination ratio was measured at 24-hour intervals for 5 days at 30°C in the dark. On the 5th day, the seeds of the varieties having poor germination were taken out from the deoxidized water and put on wet filter papers in petri dishes including sufficient air oxygen. After this, the measurement was continued at 30°C in the dark.

Investigation of the post-germination growth in water

The germination test for the 15 varieties of 0. *sativa* was carried out in the distilled water and deoxidized water for 8 and 6 days, respectively. Sixteen seeds of each variety were sampled from the beaker on the 2nd, 4th, 6th and 8th day after the test beginning. Of the 16 seeds, 8 seeds were used for measurement of the dry matter weights of endosperm (ESW) and growing part (GPW; radicle+plumule+embryo) in the seeds. The initial value of GPW is the weight of embryo. The other 8 seeds were used for measurement of the respiration rate as mentioned below.

Measurement of dark respiration rate

The respiration rate was measured on each variety at 30°C at 2 -day intervals. After the air contained in seeds or plants was sufficiently evacuated, the oxygen absorption rate of the materials was measured with an oxygen electrode (Clark type, Rank Brothers, Britain). When the plants grew too large to be contained in the electrode cell, the materials were cut into 2 or 3 segments and used for measurement. The respiration rate was determined as the value per unit weight of plant or grain without hull.

Experiment 2. Observation of the germination in paddy soil

Among the 15 varieties of 0. *sativa*, 5 varieties (Nipponbare, Razza 77, Calrose 76, ST Kinandan Patong and Akenohoshi) were used here as materials for investigation. Arable soil from the experimental paddy field of Kyushu University was well mixed and stirred with water. The mud was stuffed up to the 10cm level below the mouth edge of a 500ml beaker. Three days after this, 16 seeds of each variety were placed at lcm, 3cm and 5cm depth in the mud, after this water was filled up to about 10cm in depth above the soil surface. The soil temperature was maintained at 30°C for 9 days. The unsown plot was provided as a control.

Nine days after the sowing, the seeds or plants were washed out from the soil to measure GPW, ESW, and shoot and root length. The oxidation-reduction potential in the soil was periodically measured with a oxidation-reduction potential meter (Hitachi Horiba 6810, Japan).

RESULTS AND DISCUSSION

The germination ratio was measured on the 17 varieties in the control (distilled water) and treatment (deoxidized water) plots. The vigor of seed germination is variable with seed-storing period and environmental condition, as explained in relation to seed dormancy and germination inhibitors (Come and Thévenot, 1982). **To** keep the original

Table 1. Variation in germination ratio (%) of 17 varieties placed in the distilled water (control) and deoxidized water.

		Treatment												
		(A) Distilled water					(B) Deoxidized water						Air supply	
Var.		Day1	2	3	4	5	1	2	3	4	5	6	7	8
Ake.	(J)	0	100				0	0	100					
Kin.	(J)	0	100				0	75	97	100				
Kos.	(J)	0	31	100			0	0	84	100				
Nip.	(J)	0	100				0	81	100					
Raf.	(It)	0	94	100			0	97	100					
Raz.	(It)	0	44	88	94	94	0	16	100					
Ron.	(It)	0	44	81	88	94	0	72	91	100				
Cal.	(U)	0	100				0	88	97	97	100			
Car.	(U)	0	100				0	100						
Sun.	(U)	0	100				0	97	100					
AS5	(In)	0	31	63	69	81	0	0	28	56	75	84	91	91
BL.	(In)	0	81	100			0	19	75	81	84	84	88	88
ST.	(In)	0	100				0	31	94	97	97	97	97	97
Mil.	(K)	0	100				0	22	84	100				
suw.	(K)	0	100				0	0	97	100				
*G107	(N)	0	38	94	100		0	0	0	3	6	66	88	94
*G150	(S)	0	19	44	69	81	0	0	0	0	19	31	31	63

(A); The seeds were placed in the distilled water containing oxygen for 5 days.
(B); The seeds were placed in the deoxidized water until the 5th day, then put on a wet filter paper during the 6th to 8th day.
Ake., Akenohoshi; Kin.,Kinmaze; Kos., Koshihikari; Nip.,Nipponbare; Raf.,Raffaello; Raz.,Razza77; Ron.,Roncarolo; Cal., Calorina; Car., Calrose76; Sun., Sun Bennet; BL.,BL Kentan Nangka; ST.,ST Kinandan Patong; Mil.,Milyang28; Suw., Suwon258.
(J), Japan; (It), Italy; (U), USA; (In), India; (K), Korea; (N), Nigeria; (S), Senegarl.
*, *O. glaberrima*.

germination vigor, the rice seeds used here were stored at 5°C in the dark after harvest.

The germination ratios determined in the deoxidized plot are compared to those of the control plot in Table 1. In the control plot the seeds showed a high germination vigor as a whole; the germination ratio reached 100% in 9 varieties of O. *sativa* on the 2nd day after the test beginning, and on the 3rd day the other 3 varieties reached 100% germination. Varieties with poor germination were AS5 (O. *sativa*) and G150 (*O. glaberrima*); the germination ratios on the 3rd day were 63% and 44%, respectively.

The germination and dormancy of rice seed are regulated by the physiological processes in the embryo or endosperm and physical restriction to water absorption (Takahashi, 1985). In var. G107 and G150 (O. *glaberrima*), water absorption was strongly restricted by hulls. To promote water absorption, hulls were peeled off from the seeds in advance of the germination test. Nevertheless the germination ratio in var. G150 showed a relatively slow increase (Table 1), which suggests that the germination of this variety was restricted not only by poor water absorption but also by physiological causes.

In the deoxidized water, the germination in many of the varieties was delayed one

or two days compared to those of the control plot (Table 1). While no delay was detected for the germination in such *O. sativa* varieties as Calrose76 and Raffaello, contrary the germination ratio of var. ST Kinandan Patong (0. *sativa*) and var. G107 and G150 (*O. glaberrima*) greatly decreased under the deoxidized condition.

The seeds with poor germination were taken out from the deoxidized water on the 5th day after the test beginning, and then put on a wet filter paper. In these seeds a high recovery was found in germination vigor within 2 or 3 days (Table 1). The germination ratios of var. G107 and G150 on the 5th day were restricted to 6 and 19%, respectively, but by placing these seeds under the aerobic condition, the germination ratios came up to 94 and 63%, respectively.

0. *glaberrima* seeds have been known to have a stronger dormancy (Misro and Misra, 1969). We have frequently observed that the seeds of 0. *glaberrima* could not well germinate in the soil of low O_2 concentration. Also in this experiment the varieties of *O. glaberrima* could not well germinate in the deoxidized water, but the germination ratio, particularly in var. G107, quickly increased by replacing the seeds under the aerobic condition. This means that the seeds maintained a high potential in germination vigor during the treatment period; $Na_2S_2O_4$ having no or little toxic effects on the germination.

There was a varietal difference in germination response as mentioned above. In addition to the germination ratio, the early growth vigor after germination was also regarded as an important determinant for the growth and dry matter production at the following stage. Time courses of GPW and ESW in the control and deoxidized plots are shown in Fig. 1 (A, B, C and D). The process from water absorption to germination beginning has been divided into the three phases (Takahashi, 1962), though this initial process was not surveyed here; the investigation was made on the growth after this process.

GPW in the control plot (Fig. 1A) began to increase rapidly on the 4th day after the treatment beginning, and a similar logistic growth curve was shown in all the 5 varieties. GPW of each variety reached about 6mg/seed by the 8th day. ESW was large in var. Razza77 (28mg/seed) and those of the other 4 varieties were about 19mg /seed (Fig. 1B). ESW of these varieties decreased to 40 to 50% by the 8th day, and the ratio of transformation (Increase in GPW/Decrease in ESW) was 55 to 60%.

A significant difference was found in GPW for the varieties in the deoxidized water plot (Fig. 1C). GPW of var. Razza77 and Calrose76 increased with time for 6 days, and that of var. Nipponbare had a relatively rapid increase until the 4th day, while the growth of var. ST Kinandan Patong was strongly restricted. Var. Razza77 and Calrose76 reached about 0.7mg/seed on the 6th day; this value was about 20% of those of the control plot. Three varieties (Akenohoshi, Nipponbare and ST Kinandan Patong) showed almost 100% in germination ratio as shown in Table 1, but had no continuous increase in GPW after germination. ESW of var. Razza77 and Calrose76 reduced by 10 to 20% for 6 days, but those of the other 3 varieties had a less reduction with time (Fig. 1D). Of the 15 varieties of 0. *sativa* used here, the 10 varieties other than the 5 varieties shown in Fig. 1 had no continuous increase in GPW in the deoxidized water and gradually died during the 8 days.

The energy necessary to initiate germination and early growth is produced from accumulated carbohydrate in the endosperm mainly through the biochemical system

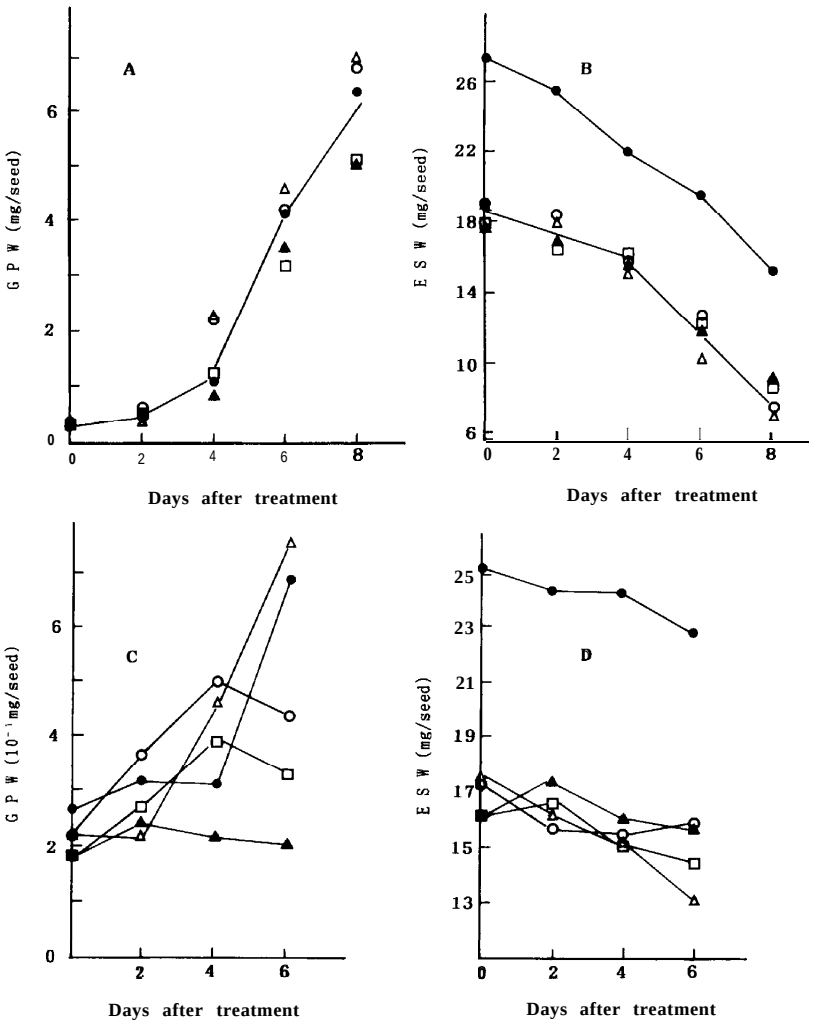

Fig. 1. Time course of GPW and ESW in the distilled water (A and B) and deoxidized water (C and D). ○, var. Nipponbare; ●, var. Razza77; △, var. Calrose76; A, var. ST Kinandan Patong; ❑ , var. Akenohoshi.

from glycolysis to tricarboxylic acid (TCA) cycle. Adenosine triphosphate (ATP) level in the seeds increases rapidly about 24 hours after the germination beginning, then reaching a maximal level about 10 hours later (Ching and Kronstad, 1972). The energy producing system is expected to operate smoothly in the seeds which have been placed in the distilled water containing oxygen. On the other hand, in the deoxidized water the aerobic respiration was stopped, and the energy used for growth is considered to be produced mainly by the anaerobic metabolism from glycolysis to fermentation.

It is expected from the results shown in Fig. 1 that the energy production capacity

in the anaerobic metabolism was higher in both var. Razza77 and Calrose76 than in the other varieties, and based on the produced energy, both varieties could maintain growth in the deoxidized water. The anaerobic metabolism is a complicated process composed of ten and several enzymes. Under the anaerobic condition the alcoholic fermentation process has been known to operate using the final product (pyruvate) of glycolysis (Lambers, 1985). A high energy productivity in the deoxidized water is probably due to a higher enzymatic activity in the alcoholic fermentation process.

Figure 2 (A and B) shows the respiration rate of 5 varieties of 0. *sativa* tested in the distilled and deoxidized water. In the distilled water, the respiration rate of these varieties showed a roughly similar change with time; increasing after the germination beginning and having a peak on the 2nd to 4th day (Fig. 2A).

On the other hand, there was a large difference in it among the varieties placed in the deoxidized water (Fig. 2B). The increase in respiration rate was not found in varieties such as Akenohoshi, Nipponbare and ST Kinandan Patong. While var. Razza77 and Calrose76 maintained an increasing trend in respiration rate with day (Fig. 2B), both which also showed a continuous increase in GPW in the deoxidized water (Fig. 1C). It should be taken into account that the respiration rate was measured in water containing oxygen immediately after the seeds or plants were taken out from the deoxidized water. That is, although the aerobic respiration was completely stopped in the deoxidized water, yet the two varieties, Razza77 and Calrose76, could began aerobic respiration directly after oxygen was supplied.

The relationship between the initial embryo ratio (embryo / embryo+endosperm) measured at the test beginning and the increase ratio of GPW (GPW / the initial weight

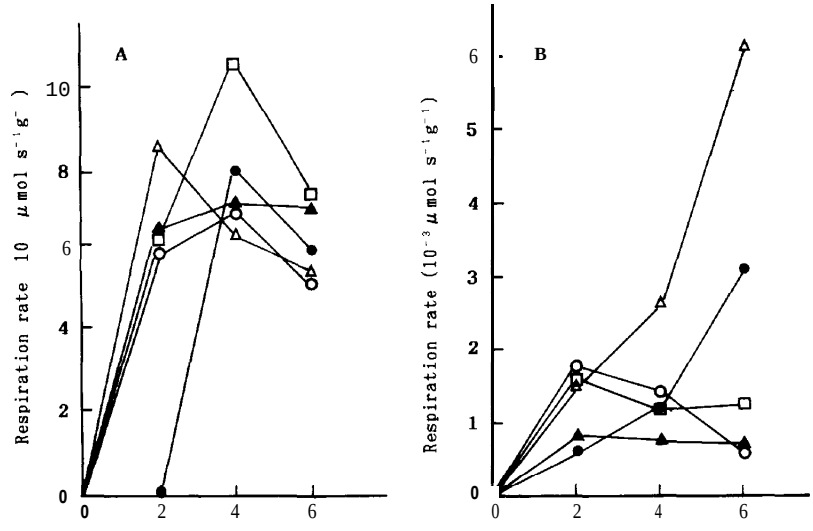

Fig. 2. Varietal difference in respiration rate of seeds (plants) germinated and grown in the distilled water (A) and deoxidized water (B). See Fig. 1 for the symbols.

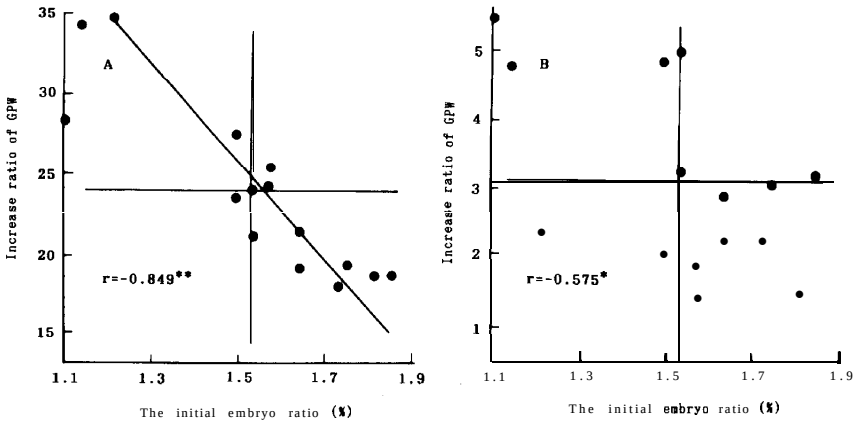

Fig. 3. Relationship between the increase ratio of GPW and the initial embryo ratio. (A); The seeds were placed in the distilled water for 8 days. (B); The seeds placed in the deoxidized water during the first 5 days were put under the aerobic condition during the next 3 days.

of embryo) measured on the 8th day is shown in Fig. 3 (A and B). Figure 3 A is the result obtained with the seeds which were placed on a wet filter paper through 8 days, and in Fig. 3B the seeds were submerged in the deoxidized water during the first 5 days, then taken out and put on a wet filter paper during the next 3 days. There was a negative relationship, r= -0.849, $p<0.01$, in Fig. 3A. This indicates that the varieties with small embryo (or larger endosperm) had a vigorous growth. Contrary, the dots shown in Fig. 3B distributed in a wide range, which indicates that the embryo ratio is not always a determinant for the vigorous germination and growth in the deoxidized water. This genetical variation may be caused, as mentioned above, by difference in enzymatic activity of the anaerobic respiration metabolism.

To examine the germination characteristics of varieties in soil, the oxidation - reduction potential, GPW, transformation ratio and shoot length were measured on the seeds placed in the flooded paddy soil; these are shown in Fig. 4 (A, B, C and D), respectively.

The potentials were 70 to 150mv at 1 to 5cm soil depth just before sowing, then decreased to negative levels in both unsown and sown plots (Fig. 4A). The potentials in the unsown soil are measured as control values. The measurements in the sown soil were about 100mv lower than the control values, and the soil in the'vicinity of seeds was more strongly reduced.

Var. Nipponbare and Akenohoshi had a vigorous growth when the seeds were sown at 1cm depth, but their GPW greatly decreased with depth from 1 to 5cm (Fig. 4B). While the growth vigor of var. Razza77 and Calrose76 did not so much decrease with depth. Both varieties are expected to have a higher tolerance against the anaerobic conditions (the deoxidized water and the flooded paddy soil). The growth of var. ST Kinandan Patong was strongly restricted even at 1cm depth in the soil.

A large varietal difference was detected in transformation ratio (Fig. 4C). Var. Razza77 and Calrose76 maintained a relatively high ratio at each depth. The

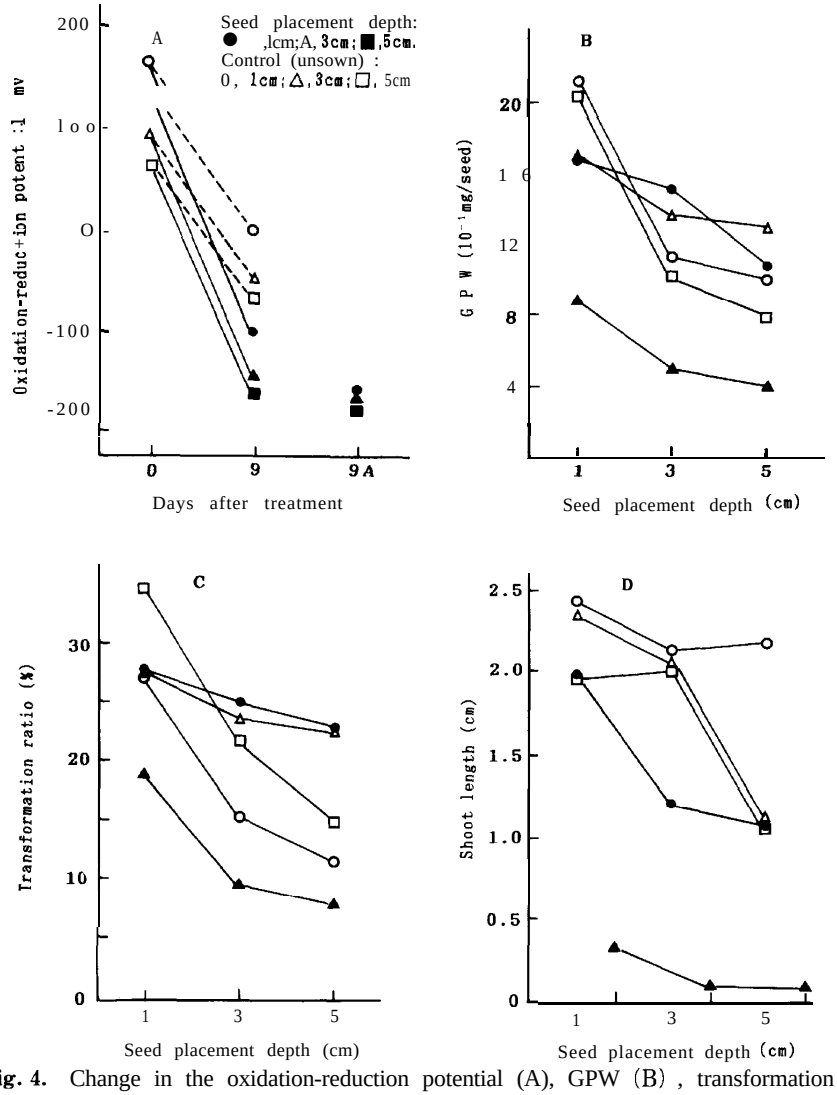

Fig. 4. Change in the oxidation-reduction potential (A), GPW (B), transformation ratio (C) and shoot length (D) with seed placement depth in the soil. The oxidation-reduction potentials shown on the 9A of Fig. A were measured in the vicinity of seeds in the soil on the 9th day after the treatment beginning. The measurements of Fig. B, C and D were obtained on the 9th day. See Fig. 1 for the symbols shown in Fig. B, C and D.

transformation ratios of the 5 varieties measured here were considerably lower than those (55 to 60%) in the distilled water. The varieties with vigorous growth in the soil also had a higher ratio in transformation. This means that the energy generated from the accumulated carbohydrate of endosperm was effectively used for growth initiation of seed in these varieties. The transformation ratio is regarded as an important

criterion for selecting the genotypes with a high tolerance against the anaerobic environments which frequently occur at the germination time in the direct sowing cultivation system.

The shoot length of the 4 varieties other than var. ST Kinandan Patong was 2.0 to 2.5cm at the lcm depth placement (Fig. 4D). The elongation ability was superior in var. Nipponbare.

The response of germination and early growth to the deoxidized water (Fig. 2) had a good agreement with that examined in the flooded paddy soil (Fig. 4). The germination test by $Na_2S_2O_4$ solution is recommended as a method for selecting the rice genotype expected to have a vigorous germination and early growth in the direct sowing cultivation system.

REFERENCES

Ching, T. M. and W. E. Kronstad 1972 Varietal differences in growth potential, adenylate energy level, and energy charge of wheat. Crop *Sci.*, 12: 785-789

Ching, T. M. 1982 Adenosine triphosphate and seed vigor. *In* "The physiology and biochemistry of seed development, dormancy and germination." ed. by A. A. Khan, Elsevier Biomedical Press, Amsterdam, pp. 487-506

Come, D. and C. Thévenot 1982 Environmental control of embryo dormancy and germination. In "The physiology and biochemistry of seed development, dormancy and germination." ed. by A. A. Khan Elsevier Biomedical Press, Amsterdam, pp. 271-298

Hagiwara, M., M. Imura and S. Mitsuishi 1987 Oxidation-reduction state in the vicinity of the paddy rice seed sown into flooded soil. *Jpn. J. Crop. Sci.*, 56: 356-362 (in Japanese)

Misro, B. and P. K. Misra 1969 Certain consideration on seed dormancy in rice. *Oryza*, 6: 18-22

Lambers, H. 1985 Regulatory aspects of respiration in vivo. *In* "Higher plant cell respiration" Encyclopedia of plant physiology. New Series 18. ed. by R. Douce and D. A. Day, Springer -verlag, Berlin, pp. 438-446

Ota, Y. and M. Nakayama 1970 Effect of seed coating with calcium peroxide on germination under submerged condition in rice plant. *Jpn. J. Crop. Sic., 39:* 535-536 (in Japanese)

Takahashi, N. 1953 Studies on the germination of rice seed. Part 2. The carbohydrate metabolism and the development of plumule or radicle. *Bull. Inst. Agy. Res. Tohoku Univ.*, 5: 249-267 (in Japanese)

Takahashi, N. 1962 Physicogenetical studies on germination of rice seeds with special reference to its genetical factors. *Bull. Inst. Agy. Res. Tohoku Univ.*, 14: l-87 (in Japanese)

Takahashi, N., T. Kato, M. Tsunagawa, N. Sasaki and Y. Kitahara 1975 Mechanisms of dormancy in rice seeds. II New growth inhibitors, momilactone-A and -B isolated from the hull of rice seeds. *Japan. J. Breed., 26:* 91-98

Takahashi, N. 1985 Inhibitory effects of oxygen on the germination of *Oryza sativa* L. seeds. *Ann. Bot., 55: 597-600*