Annals of Botany **79**: 391–395, 1997

The Effects of Ethanol on the Germination of Seeds of Japonica and Indica Rice (*Oryza sativa* L.) under Anaerobic and Aerobic Conditions

KAZUMITSU MIYOSHI* and TADASHI SATO†

The Institute for Genetic Ecology, Tohoku University, Katahira, Sendai, 980–77 Japan

Received: 22 July 1996 Accepted: 25 October 1996

An examination was made of the effects of ethanol at 0·2–6·0% (v/v) on the germination, under aerobic conditions, of intact and dehusked seeds of indica rice (cv. Assam IV), which had been harvested 14, 21 and 28 d after anthesis, and of the japonica rice (cv. Sasanishiki), which had been harvested 30 and 60 d after anthesis. The inhibition of germination caused by dehusking japonica rice was overcome by 0·5–5% ethanol, with maximum germination (frequently 100%) achieved at 3–5% (30 d after anthesis) or 1–4·5% (60 d after anthesis) ethanol. Further increases in the ethanol concentration reduced germination. The germination of dehusked indica rice was slightly inhibited at 0·5 and 1% ethanol, whilst the promotion of germination by 2% ethanol increased as the seeds matured. At all harvests germination was greatest at 3% ethanol, and at 5–6% ethanol germination fell to 0%. Inhibition, no effect, or minimal stimulation of the germination of intact seeds of both japonica and indica rice by ethanol was observed at the concentrations examined. The absence of oxygen stimulated germination of dehusked japonica rice, but this germination was inhibited by ethanol. In contrast ethanol had little or no effect on the failure of dehusked indica seeds to germinate in anaerobic conditions. Thus ethanol treatment may help break the strong dormancy of dehusked seeds of indica and japonica rice. The possible role of ethanol in stimulating germination in rice is discussed.

Key words: Rice, *Oryza sativa* L., seed germination, dehusking treatment, ethanol, indica, japonica, oxygen, dormancy, germination inhibition, seed formation.

INTRODUCTION

It has previously been demonstrated that dehusking immediately after harvest stimulates the germination of seeds of indica rice but strongly inhibits that of japonica rice (Takahashi, 1985; Takahashi and Miyoshi, 1985). This intrinsic trait of japonica rice was confirmed in a comparative study of seeds from a wide range of indica and japonica rice cultivars that had been collected at the time of traditional harvest (Miyoshi, 1988; Miyoshi, Sato and Takahashi, 1996*a*) and it was unaffected by the exposure of the mother rice plants to high- or low-temperature regimes in growth cabinets (Miyoshi, Sato and Takahashi, 1996*b*). We have also reported that the inhibitory effects of dehusking on the germination of seeds of japonica rice can be overcome by anaerobic conditions, for example, by soaking the seeds in water or by removing oxygen from the surrounding atmosphere. Thus, oxygen in the atmosphere around dehusked seeds appeared possibly to be related to these inhibitory effects (Takahashi and Miyoshi, 1985). We reported recently, however, that peeling (removal of the pericarp and testa that cover the embryo) of japonica rice seeds restored the germination of seeds that had exhibited strong inhibition of germination after dehusking. Therefore, the probable extent of the availability of oxygen to intact seeds, dehusked seeds and peeled seeds did not coincide with the rank order of germination frequencies of the three forms of seeds (Miyoshi and Sato, 1997).

Ethanol has been reported to have stimulatory effects on the germination of seeds of many plant species (Taylorson and Hendricks, 1979; Bewley and Black, 1982). To our knowledge, there is, however, only one preliminary report of the effects of ethanol on the germination of cultivated rice seeds (Ikeda, 1963). Thus, the responses to ethanol of seeds of both indica and japonica rice, which have intrinsic differences in germinability upon dehusking, remain to be characterized.

The objectives in the present study were: (*a*) to determine the effects of ethanol at different concentrations on the germination, in air, of seeds of indica and japonica rice that had been harvested at different times during the formation of seeds; and (*b*) to elucidate, under aerobic and anaerobic conditions, the effects of ethanol at the concentrations that were found to be optimal for stimulation of the germination of rice seeds.

MATERIALS AND METHODS

Plant materials

The two cultivars of rice (*Oryza sativa* L.) used in this study had previously been classified as indica or japonica, according to the criteria described by Ueno, Sato and Takahashi (1990). Plant materials were cultured in the

* Present address: Sakata Seed Corp. Kakegawa Breeding Station, Kakegawa, Shizuoka, 436–01 Japan

† For correspondence.

0305-7364/97/040391+05 $25.00/0 bo360364

manner described previously (Miyoshi and Sato, 1997). The day when the majority of spikelets in the primary rachis branches, that arose from the upper half of panicles, had flowered on 5 previous consecutive days was recorded and the day 2 days prior to this date was taken as the day of anthesis.

Harvest and storage of seeds

The first serial seed harvest was conducted half way through the time of the traditional harvest in paddy fields. Seeds of the indica cultivar Assam IV were harvested 14, 21 and 28 d after anthesis and those of the japonica cultivar, Sasanishiki, were harvested 30 and 60 d after anthesis. Seeds of primary rachis branches on the upper half of approx. 100 panicles were removed. Seeds were then dried rapidly in a drying room at a temperature of 18–22 °C. The water content of seeds was determined by the previously reported procedure (Miyoshi *et al*., 1996*b*). Seeds with a moisture content of 10–11 % (w/d) were stored, prior to use, in airtight plastic boxes at −20 °C without a desiccant.

Treatment of seeds with ethanol at different concentrations in air

Germination tests were performed with intact and dehusked seeds. Sterilization of seeds and dehusking were performed as described previously (Miyoshi and Sato, 1997).

Seeds in each group (intact and dehusked) were placed on three layers of filter paper (Toyo no. 2; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) in a petri dish (6 cm in diameter) that contained 4·5 ml of deionized water or an aqueous solution of ethanol at different concentrations, as indicated in the text. The petri dish was sealed with two layers of Parafilm™ (American Can Company, CT, USA). The number of seeds from which a radicle or plumule of at least 2 mm in length had emerged was recorded at 24 h intervals during a 10 d incubation at 30 °C in darkness, with triplicate groups of 25 intact or dehusked seeds.

Germination of seeds under anaerobic (0 % oxygen) and under aerobic (20 % oxygen) conditions with or without ethanol treatment

Twenty-five seeds were placed in a 135 ml Erlenmeyer flask that contained three layers of filter paper and 4·5 ml of deionized water or an aqueous solution of ethanol in deionized water. The concentration of ethanol chosen was that with the greatest stimulatory effect on the germination of seeds. Three replicates were prepared for each treatment. Gases were introduced into Erlenmeyer flasks as described previously (Miyoshi and Sato, 1997). At the start of tests and after the removal of germinated seeds at 24 h intervals, gases were introduced into the flasks, via a needle connected to a needle valve, at a rate of 133 ml min^{-1} for 15 min. The number of germinated seeds was recorded daily during the 10 d incubation.

RESULTS

Effect of ethanol at different concentrations on the germination of seeds of indica and japonica rice

Figures 1 and 2 show the effects of ethanol at concentrations of 0·2–6 % (v/v) on the germination of intact and dehusked rice seeds that had been harvested at various times during the development and maturation of seeds of the indica

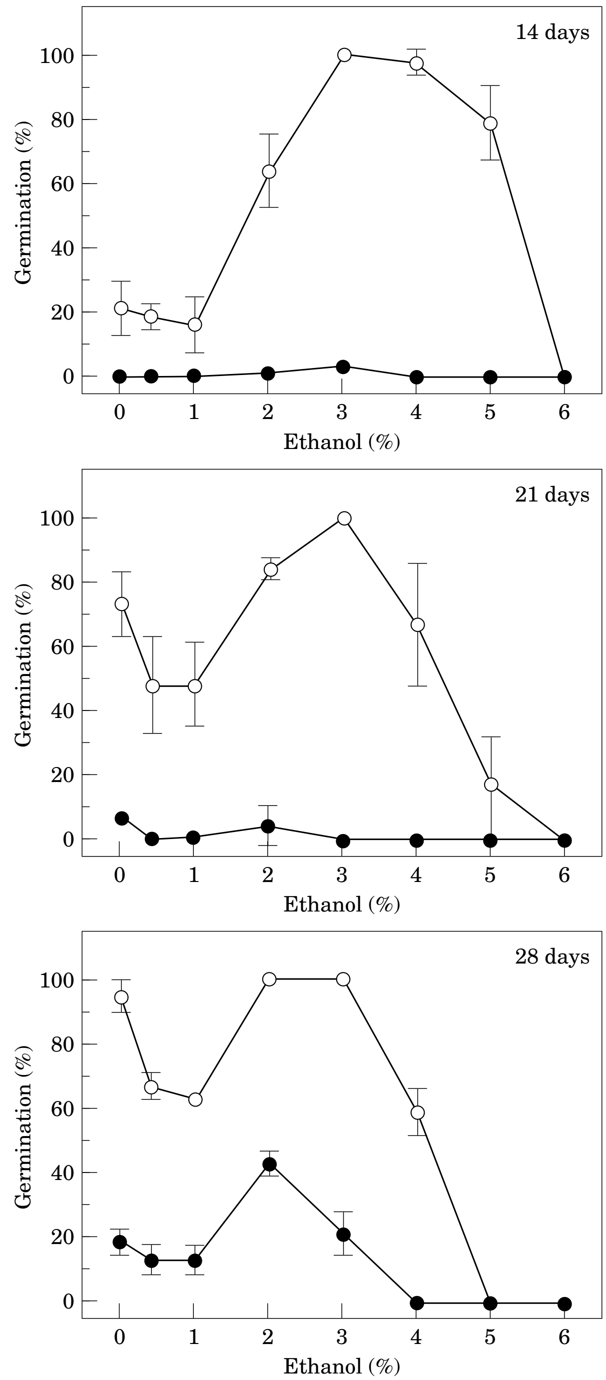

FIG. 1. The effects of ethanol at different concentrations on the germination in air of intact (●) and dehusked (○) seeds of the indica rice cultivar Assam IV, harvested 14, 21 and 28 d after anthesis.

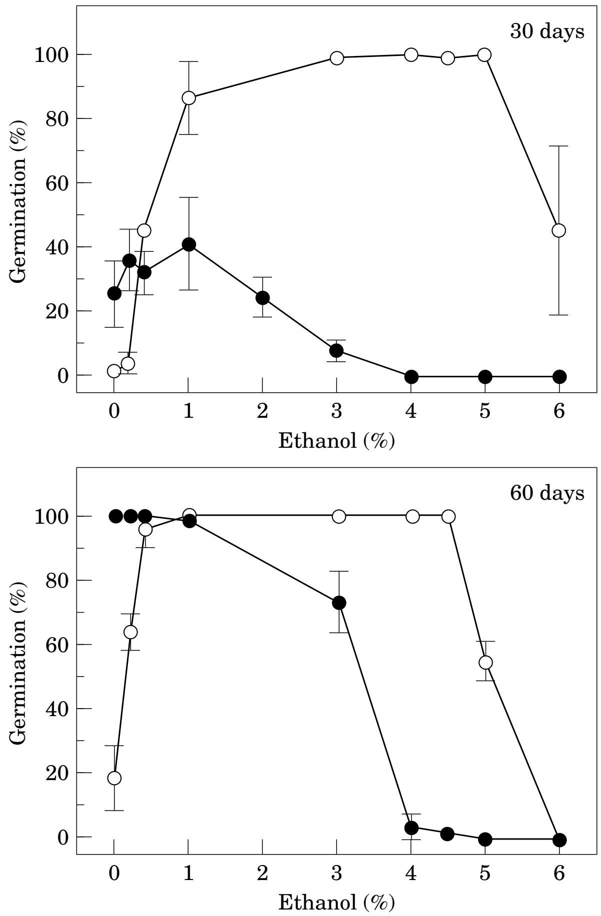

FIG. 2. The effects of ethanol at different concentrations on the germination in air of intact (●) and dehusked (○) seeds of the japonica rice cultivar Sasanishiki, harvested 30 and 60 d after anthesis.

rice cultivar Assam IV and japonica rice cultivar Sasanishiki. Of the intact seeds of indica rice, only those harvested 28 d after anthesis showed a positive response, and approx. 40 % of seeds germinated after treatment with 2 % ethanol (Fig. 1). Dehusked seeds of indica rice that had been harvested 14, 21 and 28 d after anthesis had germination percentages of 21·3–94·7 % in the absence of ethanol treatment. Germination of dehusked seeds of indica rice that had been harvested at each time point was stimulated by 2 and 3 % ethanol, with 100 % germination after treatment with 3 % ethanol. At lower concentrations of ethanol (0·4 and 1·0 %) germination of seeds harvested on all three occasions was slightly inhibited. After treatment with 6 % ethanol no germination was observed in seeds from any harvest. Treatment with 4 and 5 % ethanol gave higher germination percentages in seeds that had been harvested earlier (Fig.1).

In the case of japonica rice, no obvious promotion of germination by ethanol was observed in intact seeds harvested 30 d after anthesis. Treatment with ethanol at concentrations above 3 % had an inhibitory effect on the germination of intact seeds harvested either 30 or 60 d after anthesis (Fig. 2). The germination of dehusked seeds of Sasanishiki harvested 30 and 60 d after anthesis was greatly enhanced by ethanol and almost all of the seeds germinated after treatment with 3–4·5 % ethanol (Fig. 2).

TABLE 1. *The effects of ethanol and oxygen tension on the germination of seeds of the japonica rice cultivar Sasanishiki harvested 30 and 60 d after anthesis*

Days after anthesis	Seeds	Oxygen (%)	Ethanol (%)	Germination (%)
30	Intact	20	0·0	20·0 ± 6·5
			4·5	0·0
	Dehusked	0	0·0	97·3 ± 1·9
			4·5	0·0
		20	0·0	1·3 ± 1·9
			4·5	100·0
60	Intact	20	0·0	100·0
			4·0	0·0
	Dehusked	0	0·0	100·0
			4·0	0·0
		20	0·0	16·0 ± 5·7
			4·0	100·0

Each number represents the mean germination percentage ± s.d and was obtained from analysis with triplicate groups of 25 seeds for intact and dehusked seeds.

TABLE 2. *The effects of ethanol and oxygen tension on the germination of seeds of the Assam IV cultivar harvested 14 and 28 d after anthesis*

Days after anthesis	Seeds	Oxygen (%)	Ethanol (%)	Germination (%)
14	Intact	0	0	0·0
			3	0·0
		20	0	0·0
			3	0·0
	Dehusked	0	0	0·0
			3	0·0
		20	0	34·7 ± 1·9
			3	100·0
28	Intact	0	0	0·0
			2	0·0
		20	0	13·3 ± 3·8
			2	41·3 ± 10·5
	Dehusked	0	0	0·0
			2	10·7 ± 9·4
		20	0	96·0 ± 3·3
			2	100·0

Each number represents the mean germination percentage ± s.d. and was obtained from analysis with triplicate groups of 25 seeds for intact and dehusked seeds.

Germination of seeds under anaerobic (0 % oxygen) or aerobic (20 % oxygen) conditions with or without ethanol treatment

As shown in Table 1, under aerobic conditions (20 % oxygen tension), with no ethanol treatment, only minimal germination of dehusked seeds of japonica rice harvested either 30 or 60 d after anthesis, was observed. Treatment with 4 or 4·5 % ethanol greatly enhanced germination, and all dehusked seeds harvested at both dates germinated under aerobic conditions. Under anaerobic conditions, germination of almost all dehusked seeds occurred without any ethanol treatment. However, this enhancement of germination was eliminated by treatment with ethanol. The germination of dehusked seeds of indica rice (Table 2) that had been harvested 14 and 28 d after anthesis was enhanced by treatment with ethanol under aerobic conditions, giving germination percentages of 100 %. In anaerobic conditions, however, the dehusked indica rice failed to germinate and there was no or little (10·7 %) enhancement of germination with ethanol treatment. This was in contrast to the germination of dehusked japonica rice in these conditions (Table 1).

DISCUSSION

As reported previously, the germination of japonica rice seeds is strongly inhibited by dehusking (Miyoshi, 1988, Miysohi and Sato, 1997), but this is overcome in seeds, harvested either 30 or 60 d after anthesis, by ethanol (Table 1; Fig. 2). The germination of indica rice seeds that had been harvested 14 d after anthesis and that germinated poorly even after dehusking, was also greatly enhanced by treatment with ethanol (Table 2; Fig. 1). Stimulation of the germination of caryopses by ethanol was reported in *Panicum dichotomiflorum* (Taylorson and Hendricks, 1979) and *Avena* spp. (Adkins, Simpson and Naylor, 1984, 1985, Corbineau *et al*., 1991). Two different mechanisms by which ethanol might break dormancy have been proposed; Taylorson and Hendricks (1979) suggested that the stimulatory effect of ethanol might involve modification of the properties of a membrane(s). Ethanol might also be involved metabolically in the stimulation of germination, as a respiratory substrate. It might accelerate germination by promoting the uptake of oxygen (Fidler, 1968; Adkins *et al*., 1984) and increasing levels of fructose 2,6-bisphosphate which has been suggested to stimulate glycolysis in dormant seeds of *Avena sativa* (Larondelle *et al*., 1987). In the present study, the promotive effects of ethanol on the germination of seeds of both indica and japonica rice were observed only under aerobic conditions (Tables 1 and 2). According to the different responses of seeds to ethanol under aerobic and anaerobic conditions, we can postulate that ethanol might influence a step in the metabolic pathway in rice seeds. It was suggested that ethanol might break dormancy by promoting the Krebs cycle and/or glycolysis (Adkins *et al*., 1984; Corbineau *et al*., 1991). Ethanol was also reported to induce alcohol dehydrogenase (ADH) activity and could stimulate alcohol fermentation via the action of ADH (App and Meiss, 1958; Hageman and Flesher, 1960; Rumpho and Kennedy, 1981). The inhibitory effects of dehusking on germination of seeds of japonica rice under aerobic conditions were overcome by reducing the oxygen tension in the atmosphere (Takahashi and Miyoshi, 1985; Miyoshi and Sato, 1997). In the present study, the inhibitory effects of dehusking in japonica rice were overcome by ethanol under aerobic conditions. Therefore, it is possible that ethanol might induce conditions in embryos that are physiologically similar to those induced by anaerobiosis.

Under anaerobic conditions, no promotive effects of ethanol on the germination of japonica seeds were observed. Excess ethanol might be produced endogenously, perhaps via activation of ADH, and exogenously applied ethanol might hamper germination. In indica rice, the promotive effects of ethanol under aerobic conditions might involve activation of the Krebs cycle and/or glycolysis. By contrast, under anaerobic conditions, no promotive effects of ethanol were observed (Table 2). The metabolism of dormant seeds of indica rice is locked into a pattern of aerobic respiration (Kono *et al*., 1975), under anaerobic conditions there is insufficient energy available for germination.

Dose-response curves were drawn for the effects of ethanol on the germination of seeds of indica and japonica rice (Figs 1 and 2). In dehusked seeds of both indica and japonica rice, the range of optimum concentrations of ethanol that yielded germination percentages of approx. 100 % decreased with the extent of seed development and maturation. In the case of indica rice, this range was 3–4 % ethanol in seeds harvested 14 d after anthesis, 3 % in seeds harvested 21 d after anthesis and 2–3 % in seeds harvested 28 d after anthesis. In the case of japonica rice, it was 2–5 % ethanol in seeds harvested 30 d after anthesis and 0·4–4·5 % in seeds harvested 60 d after anthesis. In our previous report we suggested that, in indica rice, the development of metabolic machinery during formation of seeds might be similar to that during the acceleration of after-ripening by an elevated temperature, with aerobic respiration developing first (Miyoshi and Sato, 1997). Therefore, at least in indica rice, the change in optimum concentrations for induction of the stimulatory effects of ethanol on germination might coincide with the development of the metabolic machinery during the development and maturation of seeds.

Further experiments are needed to examine the nature of the metabolic activity, such as glycolysis and operation of the tricarboxylic acid cycle, in rice seeds. Such studies might help to explain the stimulatory effects of ethanol on the germination of japonica rice seeds, which was strongly inhibited by dehusking.

Seed dormancy in cultivated rice can be a problem in some cases, such as post-harvest, in seed testing, in the production of two or three crops per year, and in plant breeding (Ellis, Hong and Roberts, 1983). In the present study, in both indica and japonica rice cultivars, the germination of all seeds harvested at any point during their formation, was achieved by a combination of dehusking and the application of ethanol under aerobic conditions. In recent preliminary experiments, germination of seeds of the indica rice cultivar Surjamkhi, which exhibits strong dormancy (Amemiya, Akemine and Toriyama, 1956; Miyoshi, 1988), was also induced by the procedures used in the present study. Hence, the application of ethanol to dehusked seeds under aerobic conditions appears to be a simple and efficient procedure for breaking dormancy of rice seeds and should be applicable to rapid-cycling programmes.

ACKNOWLEDGEMENT

The authors thank Dr Norindo Takahashi, Professor Emeritus of Tohoku University, for his valuable suggestions during this study.

LITERATURE CITED

Adkins SW, Simpson GM, Naylor JM. 1984. The physiological basis of seed dormancy in *Avena fatua* VI. Respiration and the stimulation of germination by ethanol. *Physiologia Plantarum* **62**: 148–152.

Adkins SW, Simpson GM, Naylor JM. 1985. The physiological basis of seed dormancy in *Avena fatua*. VII. Action of organic acids and pH. *Physiologia Plantarum* **65**: 310–316.

Amemiya A, Akemine H, Toriyama K. 1956. The first germinative stage and varietal differences in growth response of cultured embryos in rice plant. *Bulletin of the National Institute of Agricultural Sciences*, **D6**: 41–60.

App AA, Meiss AN. 1958. Effect of aeration of rice alcohol dehydrogenase. *Archives of Biochemistry and Biophysics* **77**: 181–190.

Bewley JD, Black M. 1982. *Physiology and biochemistry of seeds in relation to germination. Vol. 2, Viability, dormancy and environmental control*. New York: Springer-Verlag.

Corbineau F, Gouble B, Lecat S, Come D. 1991. Stimulation of germination of dormant oat (*Avena sativa* L.) seeds by ethanol and other alcohols. *Seed Science Research* **1**: 21–28.

Ellis RH, Hong TD, Roberts EH. 1983. Procedures for the safe removal of dormancy from rice seed. *Seed Science and Technology* **11**: 77–112.

Fidler JC. 1968. The metabolism of acetaldehyde in plant tissues. *Journal of Experimental Botany* **58**: 41–51.

Hageman RH, Flesher D. 1960. The effect of anaerobiotic environment on the activity of alcohol dehydrogenase. *Archives of Biochemistry and Biophysics* **87**: 203–209.

Ikeda M. 1963. Studies on the viviparous germination of rice seed. *Bulletin of the Faculty of Agriculture, Kagoshima University* **13**: 89–115.

Kono K, Takeuchi S, Kawarada A, Ota Y. 1975. Anaerobic respiration in the dormancy of rice seed. *Proceedings of Crop Science Society of Japan* **44**: 194–198.

Larondelle Y, Corbineau F, Dethier M, Come D, Hers H-G. 1987. Fructose 2,6-bisphosphate in germinating oat seeds. A biochemical study of seed dormancy. *European Journal of Biochemistry* **166**: 605–610.

Miyoshi K. 1988. Physiological study of germinability between Japonica and Indica rice seed. 1. Differences in germinability observed at harvest time among ecotypes and cultivars. *Bulletin of Institute of Agricultural Research of Tohoku University* **39**: 37–41.

Miyoshi K, Sato T, 1997. Removal of the pericarp and testa of seeds of japonica and indica rice (*Oryza sativa*) at various oxygen concentrations has opposite effects on germination. *Physiologia Plantarum* **99**: 1–6.

Miyoshi K, Sato T, Takahashi N. 1996*a*. The inhibitory effects of dehusking on germination of japonica rice seed during development and maturation. In: Noda K, Mares DJ, ed. *Pre-harvest sprouting in cereals 1995*, 371–376. Center for Academic Societies Japan, Osaka (CASJO), Japan.

Miyoshi K, Sato T, Takahashi N. 1996*b*. Differences in the effects of dehusking during formation of seeds on the germination of seeds of indica and japonica rice (*Oryza sativa* L.). *Annals of Botany* **77**: 599–604.

Rumpho ME, Kennedy RA. 1981. Anaerobic metabolism in germinating seed of *Echinochloa crus-galli* (barnyard grass). Metabolism and enzyme studies. *Plant Physiology* **68**: 165–168.

Takahashi N. 1985. Inhibitory effects of oxygen on seed germination in rice. *Annals of Botany* **55**: 597–600.

Takahashi N, Miyoshi K. 1985. Inhibitory effects of oxygen on seed germination as a specific trait of Japonica rice, *Oryza sativa* L. *Japanese Journal of Breeding* **35**: 383–389.

Taylorson RB, Hendricks SB. 1979. Overcoming dormancy in seeds with ethanol and other anesthetics. *Planta* **145**: 507–510.

Ueno K, Sato T, Takahashi N. 1990. The indica-japonica classification of Asian rice ecotypes and Japanese lowland and upland rice (*Oryza sativa* L.). *Euphytica* **46:** 161–164.