

Article

Factors Determining Genotypic Variation in the Speed of Rice Germination

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Abstract: Rapid germination is important for both direct seeded rice in the field and for the production of germinated brown rice for healthy food. This study aims to evaluate genotypic differences in germination speed and identify characteristics that determine germination speed. Seven experiments were conducted to determine (i) the impact of dehulling on water absorption and germination, (ii) variety consistency in germination speed across crops grown in three years, and (iii) the effect of grain size. Germination speed in both paddy rice and dehulled brown rice was significantly correlated with grain moisture content at early stages of soaking, however significant interaction of genotype and grain type (paddy and brown rice) existed and varieties differed in their response to dehulling. Germination speed of grain from crops exposed to water deficit in the field was slightly slower than those with higher water supply. Sherpa/IRAT109 genotypes with smaller grain size tended to germinate faster than larger grain, however no significant effect of grain size existed among diversity set varieties. It was concluded that genotype ranking in germination speed was consistent across years and water availability conditions, and that barriers to water absorption in hull and pericarp were important determinants of germination speed. The existence of genotypic variation in germination speed has management implications for both field crop establishment and paddy germination in food processing.

Keywords: rice; germination; brown rice; varieties; water absorption; hull



Citation: Chao, S.; Mitchell, J.; Fukai, S. Factors Determining Genotypic Variation in the Speed of Rice Germination. *Agronomy* **2021**, *11*, 1614. <https://doi.org/10.3390/agronomy11081614>

Academic Editors: Petr Smýkal and Juan Pablo Renzi

Received: 22 June 2021

Accepted: 11 August 2021

Published: 13 August 2021

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1. Introduction

High germination percentage and rapid germination is important to ensure good crop establishment in the field in direct seeded rice and also for production of germinated brown rice as a healthy food. The method of crop establishment has changed from traditional manual transplanting of seedlings to direct seeding in many countries in recent years due to rising production costs and scarcity of water and labor [1,2]. Generally, crop establishment may be poorer in direct seeded fields. To achieve good crop establishment, seeds need to be of high quality with high germinability and rapid germination, and may also require pre-germination [3]. Varieties with rapid germination are required for early vigor, particularly when early season drought may affect grain yield and also in weedy fields [4–6].

Both paddy rice and brown rice (dehulled paddy) can be germinated to obtain germinated brown rice which has been found to have greater health benefits and softer texture than brown rice. Germination level of 70% is the minimum required to produce germinated brown rice [7]. Germinated brown rice has been suggested to be beneficial in preventing some lifestyle diseases such as dietary obesity, hyperlipidemia and hypertension as well as reducing the risk of some terminal illnesses such as cancer, diabetes, cardiovascular disease, and Alzheimer's disease [8]. Other physiological effects of germinated brown rice include prevention of blood cholesterol amplification, enhancement of liver and kidney functions, improvement from stroke and alleviation of emotional unrest [9]. Results from animal experiments and clinical trials revealed that germinated brown rice is a potential

antidiabetic functional food that could maintain health and prevent complications in type 2 diabetes by providing a steady supply of bioactive compounds [10]. Germination speed is important in minimizing the time to achieve germinated brown rice in food processing [11].

The germination process of rice grain starts with water uptake, which consists of three phases including rapid, plateau and post-germination stages [12]. At the first stage, rice grains absorb water rapidly in the first 18 h after soaking, and the grain moisture content increases gradually to 25–35% and then plateaus [13]. The absorption of water at plateau stage is slow but the uptake increases rapidly again after the grains have germinated. Water infiltrates into paddy through the openings of fibrous cells in the hull and reaches the space between the hull and the pericarp [14]. The water is then absorbed through the pericarp and the absorbing quantity varies upon the location of different parts of brown rice. The absorption of water is greater at the embryo than at any other part of the grain. The absorption pathway was demonstrated by Horigane et al. [15] with two rice varieties, Koshihikari and Yamadanishiki, using three-dimensional gradient echo magnetic resonance imaging method. Rice genotype, germination conditions and type of stored carbohydrate reserves affected the duration and time scale of each stage in water uptake. El-Hendawy et al. [12] reported significant genotypic variation in water uptake in aerobic and anaerobic conditions in 58 contrasting varieties from the IRRI collection. Water uptake rate may be affected by grain size; smaller grains often have faster increase in grain moisture content. For example, Roy et al. [16] using the smallest and largest grains of rice cultivar BR1 with an almost three-fold difference in size, found that the smallest grain class completed imbibition process earlier and started germination earlier than the largest, although the largest grain class had higher germination percentage than that of the smaller grain.

The germination process is activated when grains have reached a certain moisture content. Grains are able to germinate at a moisture content of 25% and the most suitable condition for rice is approximately 30% [17]. First, the embryo swells and grows, and hydrolysis of carbohydrate takes place. Then, the hydrolysates from the endosperm reserves are translocated to the embryo and the new cell materials are synthesized, which mark the germination of grain. Morphologically, the growth stage is marked by the active growth of the radicle and plumule after germination. Unlike most cereals, rice belongs to a group of plants that can germinate and grow in conditions of anoxia or submergence [18]. The growth of the radicle is predominant when grains are germinated in aerobic conditions, whereas the growth of the coleoptile is predominant in oxygen restricted submergence conditions. In the complete absence of oxygen, there was only a 10% reduction in germination percentage in rice, while wheat germination was inhibited [17].

Common measures used to quantify the germination process in rice include the germination percentage, germination time and germination rate. The definition of terminology used in the present work is based on the review by Ranal and Santana [19]. The germination percentage refers to the germinability or germination capacity of grain expressed as the proportion of germinated grains. Germination time refers to the time when a certain percentage of grain is germinated; for example, germination time T_{70} is the time taken from the start of soaking to 70% of the grain germinated. Germination rate refers to mean number of grain germinated per unit time, and is calculated using the following formula [20]:

$$Rg = \sum_{i=1}^n \frac{Si}{Hi}$$

where Rg = the germination rate (grain/h), Si = number of germinated grains at hours of counting (grain), Hi = number of hours at which grains were counted (h), n = time of final counting (h).

Germination of rice grain is affected by the prevailing environmental conditions of temperatures [20,21], salinity [22], and moisture stress [23], and varies among genotypes in their response to these adverse conditions. The optimal temperature for maximum germination percentage and highest germination rate was reported at 30–33 °C [20,21].

In their study of 13 rice genotypes from Bangladesh, Ali et al. [21] noted that genotypes and grain physiological quality influenced the final germination and germination rate at low temperature. Among 68 mostly indica varieties, Krishnasamy and Seshu [23] reported that the rate of germination differed among varieties following moisture stress, under low temperature (15 and 20 °C) and also with aging. Singh and Singh [24], studying the germination response to moisture stress in four varieties of IR-28, Jaya, T-23 and IR-8, found that the germination decreased progressively in response to osmoticum solutions of -0.3 to -1 MPa, and that the decrease in germination at -0.3 MPa was smaller in IR-28 than in the others. Deka and Baruah [25] reported that without water stress, all 10 Indian rice varieties studied reached a germination percentage above 80% but at -0.3 MPa water stress, 8 varieties had germination above 70% while at -1 MPa, only 4 varieties were able to germinate. Hakim et al. [26], studying the effect of salinity on germination in 12 Bangladeshi and Malaysian varieties, found that when the grains were germinated in distilled water, all varieties had germination above 90% and that the difference between the variety with the highest and lowest germination was 7%. When grains were treated with salt concentration of 4 dS/m, only half of all varieties had germination above 90% and the varietal germination difference was 29%. At a salinity of 16 dS/m, only three varieties reached a germination above 50% and the germination difference was 52%.

While variety differences in germination have been examined in rice under unfavorable conditions, there are only limited studies that show genotypic variation in germination speed under favorable temperature and water conditions [23]. Finding varieties with rapid germination and understanding the factors determining genotypic variation is important for improvement in direct seeded rice production, and also for germinated brown rice processing. The present work aimed to determine genotypic variation in germination speed and considered three aspects: importance of the hull as a barrier for water absorption and germination speed, genotypic consistency for crops grown across different years, and the effect of grain size. Germinated brown rice could be produced from paddy or from dehulled brown rice, and the examination of germination speed of these two types of germinated brown rice among different varieties also provided an understanding of the importance of the hull as a barrier for germination of paddy rice.

2. Materials and Methods

2.1. Genetic Material

In the series of experiments reported in this paper, the majority of the experiments included a standard set of 8 rice varieties (Table 1). They consist of standard Australian and international varieties selected from across the grain types and also included 2 glutinous genotypes, with 2 of the 8 considered Indica as opposed to temperate Japonica. Among 6 non-glutinous varieties, amylose content in brown rice is considered intermediate in Doongara and low in the others. The textural property in milled rice was determined by instrument and a human panel (Yanco Agricultural Institute). The amylose content in brown rice was used to correlate with germination speed, and the changes of amylose after germination were not determined.

Table 1. Characteristics of selected rice varieties used in most experiments.

Variety Name	Variety Type	Grain Type	Stickiness	Texture ¹	Amylose Content (%) ²
Doongara	Indica/Japonica	Long	Non-glutinous	Firm	24.2
Koshihikari	Japonica	Short	Non-glutinous	Soft, sticky	16.4
Langi	Japonica	Long	Non-glutinous	Soft	16.1
Reiziq	Japonica	Medium	Non-glutinous	Medium	14.4
Sherpa	Japonica	Medium	Non-glutinous	Medium	16.4
Tachiminori	Japonica	Medium	Non-glutinous	Soft	17.3
TDK8	Indica	Long	Glutinous	Soft	3.4
YRW4	Japonica	Short	Glutinous	Soft	4.9

¹ Texture classification in milled rice (Yanco Agricultural Institute); ² amylose content in brown rice (average of 3 growing seasons).

Two sets of genetic material were utilized for the evaluation of the grain size effect on germination. Set 1 consisted of 46 recombinant inbred lines (RILs) derived from Sherpa/IRAT109 population, and Set 2 consisted of 24 varieties selected from a Yanco Japonica diversity set of over 200 genotypes. Among the high early vigor and high yield varieties of the diversity set, 10 varieties with the highest and 10 with the lowest 100 grain weight were selected where there was adequate availability of grains (7 of the 8 listed in Table 1 were also included). The Sherpa/IRAT109 genotypes were used only in grain size experiments.

2.2. Source of Grains

Rice grains for soaking experiments (1a and 1b) were provided by Yanco Agricultural Institute, NSW, Australia. The grains used in all other experiments were grown at Gatton campus, the University of Queensland, Australia, in the summers of 2017–2018 (year 1), 2018–2019 (year 2) and 2019–2020 season (year 3). In year 1, two water regimes were applied, which included well-watered (72 mm/week, i.e., 3×24 mm per week) with 8 varieties and water deficit (48 mm/week, i.e., 2×24 mm per week) with 4 varieties (Doongara, Sherpa, Tachiminori, and TDK8). Only one water treatment of 72 mm/week was used in year 2 and year 3. The grain samples for the Sherpa/IRAT109 genotypes and the variety (Yanco Japonica diversity) set were taken from the grains harvested in year 3.

2.3. Laboratory Experiments

The summary of laboratory experiments in the present study are shown in Table 2. The work is divided into three parts: (i) comparison of paddy and brown (dehulled) rice germination in two experiments (1 and 2); (ii) variety consistency conducted across three years (seasons) experiments (2–4), and (iii) grain size effect was examined in 3 experiments (5–7). Experiment 2 was used to compare paddy and brown rice germination as well as variety consistency across environments. All experiments were part of a project aiming to improve the textural properties of brown rice. Some experiments seem repeated but they complement each other and some are required to assess the consistency of varieties across growing environments.

Table 2. Summary of experiments conducted in the present study.

Exp	Sub-Exp	Variety	Grain Type	Grain Source	Date	Objective
1	1a	8 diverse varieties ¹	Paddy	Yanco	Dec 2017	Determine the water absorption of paddy among different varieties
	1b	8 diverse varieties ¹	Paddy	Yanco	Dec 2017	Determine the water absorption of paddy among different varieties (repeat of 1a)
	1c	8 diverse varieties ¹	Brown rice	Gatton	May 2019	Determine the water absorption of brown rice among different varieties
2	-	10 diverse varieties ²	Paddy, brown rice	Gatton	July 2020	Determine the germination speed of paddy and brown rice among different varieties (year 3)
3	-	8 diverse varieties ¹	Paddy	Gatton	Aug 2018	Determine the germination speed of paddy among different varieties (year 1)
4	-	8 diverse varieties ¹	Paddy	Gatton	Oct 2019	Determine the germination speed of paddy among different varieties (year 2)
5	-	46 RILs ³	Paddy	Gatton	May 2020	Determine the effect of grain size on germination speed of paddy among recombinant inbred lines
6	-	11 RILs ³	Paddy	Gatton	July 2020	Confirm the grain size effect found in Exp 5 using genotypes with large variation in grain size
7	-	24 diverse varieties ⁴	Paddy	Gatton	July 2020	Determine the effect of grain size on germination speed of paddy among different varieties

¹ 8 varieties in Table 1; ² 7 varieties in Table 1 (excluding TDK8) plus IRAT109, Arlesienne and YRL24; ³ Recombinant inbred lines (RILs) derived from Sherpa/IRAT109; ⁴ 7 varieties in Table 1 (excluding TDK8) plus 4108, Arlesienne, Carnaroli, Cigalon, Darmali, Somsiah_III, IR28, IRAT109, Kinandang_Patong, Oscar, RIL266, Toro2, Vialone_Nano, YRK5, YRL24, YRM69_LT2 and YRM70.

2.3.1. Comparison of Paddy and Brown Rice Water Absorption and Germination

This section contained two experiments. The water absorption was determined in both experiments, but germination was determined only in Experiment 2.

Experiment 1: Water Absorption in 8 Varieties

This experiment was conducted to determine water absorption of different varieties with paddy and dehulled grain, and germination was not determined. Brown rice was produced by dehulling paddy using a rubber roll laboratory husker (Satake Engineering Co., Ltd., Tokyo, Japan).

The water absorption experiment of paddy (Experiment 1a,b) and brown (Experiment 1c) rice was conducted in December 2017 and in May 2019, respectively. The standard 8 varieties were used in all.

Paddy samples (10 g) of 1a and 1b were soaked in 50 mL distilled water at 25 °C and 30 °C for 24 h and 40 h, respectively. The grains were drained using steel strainer for 1 min and excessive surface water was removed using blotting paper three to four times before they were weighed. The initial weight of paddy was taken one day before the soaking. In Experiment 1a, grain weight measurements were taken every 10 min for the first hour and every 1 h for the rest of the soaking period, and for Experiment 1b, measurements were made every 5 h from 14 to 24 h of soaking and then every 2 h until the end of the observation. Experiment 1a had one replicate and Experiment 1b had 3 replicates. However, the results of Experiment 1a were verified as the measurements recorded between common times of measurement with Experiment 1b, which were consistent with a correlation coefficient of 0.996 ***.

In Experiment 1c, 10 g of brown rice was soaked in 100 mL of distilled water at 30 °C. The grains were drained using steel strainer for 1 min and wiped of excessive surface water with blotting paper three to four times before weighing for gained weight. The initial weight of brown rice was taken one day before soaking. The weight was determined every 20 min in the first hour of soaking and then every hour until 12 h. The experiment had 3 replicates.

Experiment 2: Germination Speed of Paddy and Brown Rice in 10 Varieties

Experiment 2 was conducted in July 2020 using 10 varieties (7 from Table 1, plus IRAT109, Arlesienne and YRL24). The seven varieties were included for continuity across experiments, except TDK8 which did not produce sufficient grain. IRAT109 was included as it was a parent of Sherpa/IRAT109 RILs used in Experiments 5 and 6 (see below). Two remaining varieties (Arlesienne and YRL24) were selected based on the fact that they were the two fastest to reach 70% germination among the varieties used in Experiment 7. The paddy samples were stored at room temperature for 3 months before use. All samples were tempered at 30 °C for 3 days before determination of initial grain moisture content and water absorption test.

This experiment had 3 biological replicates, and for each replicate 50 g of brown rice and 50 g of paddy were soaked in 500 mL distilled water at 30 °C for 3 and 12 h, respectively. Soaking of brown rice commenced 9 h after soaking of paddy, to ensure both samples commenced incubation at the same time. The water was changed at 3 and 9 h of soaking to minimize the fermentation odor. The soaked grains were drained and wrapped in double layer cheesecloth, placed in a plastic container with closed lid and incubated at 30 °C. After 12 h of incubation, 20 grains were removed every 3 h for germination count. Grains were considered as germinated by the protrusion of the radicle to about 0.5–1 mm. When 13 to 15 grains germinated at the time of counting, germination time was recorded, and the time for 70% germination (T_{70}) was estimated by interpolating 2 pairs of time and germination, with one just below 70% and the other just above 70% for each variety. The germination was terminated when 70% of grains had germinated. Germination of 70% was used in the present series of experiments as that is the minimum germination required for production of germinated brown rice [7]. Germination rate was calculated

using the formula in Tilebeni et al. [20]. The initial moisture content of paddy samples was determined using the simplified oven method by Bhattacharya [27].

2.3.2. Consistency in Germination Speed of 8 Varieties Grown in 3 Years Experiments 2–4: Germination of Paddy Rice

Three germination experiments were conducted in August 2018 (Experiment 3), October 2019 (Experiment 4) and July 2020 (Experiment 2—see Section 2.3.1 for experimental details). Grain of the standard 8 varieties Doongara, Koshihikari, Langi, Reiziq, Sherpa, Tachiminori, TDK8 and YRW4 from year 1 and year 2 were used in Experiments 3 and 4 while TDK8 in year 3 was excluded from Experiment 2 due to insufficient grain. The samples for Experiments 3 and 4 were stored at 4 °C for 4 and 6 months, respectively, before use, and the samples for Experiment 2 were stored at room temperature for 3 months. Grain was then maintained at 30 °C for 3 days before each experiment.

Fifty grams of paddy for Experiment 3 and 100 g paddy for Experiment 4 were placed in a 10 × 15 cm net bag and soaked in distilled water at 30 °C for 12 h. Paddy samples were tempered at 30 °C for 4 h and 3 days for Experiment 4, respectively, before soaking. The distilled water was changed after 6 h to prevent fermentation odor. The soaked bag was left to drain on wire mesh for 2 min before spreading evenly and wrapping in damp double layer cheese cloth and placed in a 1000 mL rectangular plastic container. The container with closed lid was placed in an incubator at 30 °C (with 15 mL of additional distilled water in Experiment 4 only). After 12 h of incubation, 20 grains were randomly removed every 3 h for germination count. The germination percentage, germination time (T_{70}) and germination rate in these as well as subsequent experiments were calculated using the same method as described in Experiment 2.

2.3.3. Effect of Grain Size on Germination Speed of a Population and Diverse Varieties Experiment 5: Germination of Paddy of 46 Sherpa/IRAT109 RILs

Experiment 5 was conducted in May 2020, in which paddy samples of 46 Sherpa/IRAT109 RILs were stored at room temperature for 3 months before use. All samples were tempered at 30 °C for 3 days before determination of grain moisture content and germination test. Fifty grains of each genotype, replicated twice, were placed on two layers of Whatman paper in Petri dishes to which was added 10 mL of distilled water before incubation at 30 °C in darkness. The germinated grains were counted twice a day and continued for 5 days.

Experiment 6: Germination Speed of Selected Sherpa/IRAT109 RILs with Contrast in 100 Grain Weight

Experiment 6 was conducted in July 2020 to confirm the grain size effect found in Experiment 5. From the Sherpa/IRAT109 genotypes tested in Experiment 5, 11 genotypes were selected based on variation in 100 grain weight and included the 5 heaviest and 5 lightest 100 grain weight where there was sufficient grain available for testing. Sherpa was added as it was a parent genotype. The grain source and storage and tempering conditions before the germination test were the same as in Experiment 5, except Experiment 6 was conducted one month later.

Experiment 7: Germination of Paddy of 24 Varieties from a Diversity Set

This experiment was conducted at the same time as Experiment 2 and followed the same procedure as described for Experiment 5.

2.3.4. Data Analysis

Unless otherwise stated, all measurements of variables were conducted on 3 biological replicates from samples collected from field experiments, and all points shown in figures, except Figure 1a, are the means of 3 replications. The significant difference between means of genotypes and treatments were determined by analysis of variance (ANOVA) in a completely randomized block design using GenStat 12th Edition (VSN International

Ltd., Hemel Hempstead, UK). When appropriate, two-way ANOVA in completely randomized block design was used to determine the effect of genotype, environment (grown in well-watered conditions or water deficit conditions) and grain treatments (dehulling). Means were compared by Fisher's least significant difference (LSD) range test for $p = 0.05$. The significance of relationships between all measured variables was evaluated by Pearson's correlation analysis, two-sided tests against zero at $p = 0.05$, using GenStat 12th Edition. The significance of difference was evaluated by Student *t*-test ($p = 0.05$) when two experiments were considered.

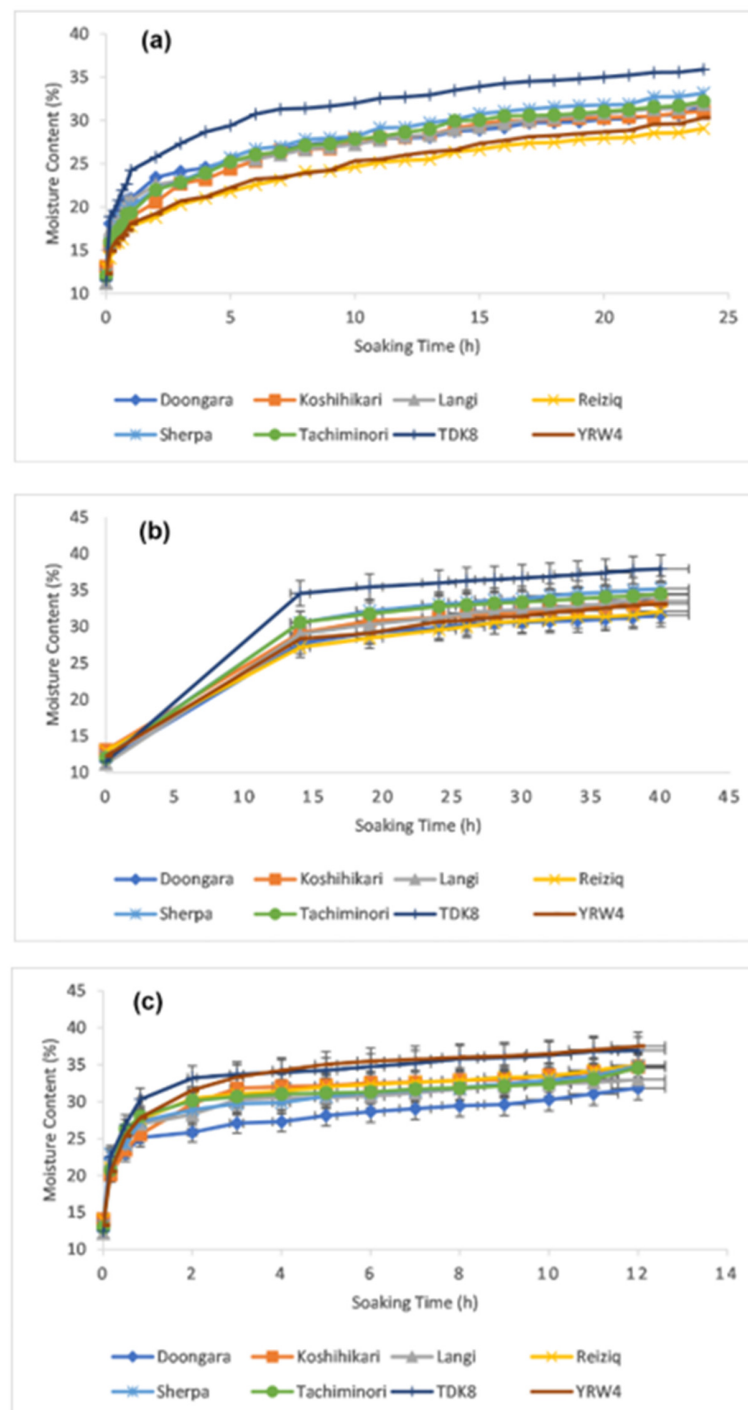


Figure 1. Grain moisture content of 8 varieties in distilled water (a) paddy rice at 25 °C for 24 h (Experiment 1a); (b) Paddy rice at 30 °C (Experiment 1b), and (c) brown rice at 30 °C for 12 h (Experiment 1c). Bars indicate standard error.

3. Results

3.1. Comparison of Paddy and Brown Rice Water Absorption and Germination

3.1.1. Experiment 1: Water Absorption in 8 Varieties

The moisture content of paddy rice (Experiment 1a) increased rapidly in the first hour of soaking, and on average reached 23% after 3 h, then increased slowly to 32% at 24 h (Figure 1a). TDK8 obtained the highest moisture content at about 36%, while Reiziq achieved the lowest at 29%. Varieties with higher moisture content after 1 h also had higher moisture at the end of the soaking period ($r = 0.91^{***}$). The results in Experiment 1b are similar to those in Experiment 1a and the relationship between moisture content after 24 h of soaking between Experiment 1a and 1b was highly significant ($r = 0.93^{***}$).

Grain moisture content of brown rice during the 12 h soaking is shown in Figure 1c. There was highly significant varietal difference in moisture content ($p < 0.001$). The mean moisture content increased rapidly reaching 31% after 3 h and then increased slowly to 35% at 12 h. Doongara and YRW4 had the lowest and highest moisture content, respectively. The varieties with higher moisture content at 3 h also had higher moisture after 12 h of soaking ($r = 0.92^{***}$). The interaction of genotype and time of soaking for moisture content was significant ($p < 0.001$).

The results of correlation analysis show that the final moisture content of brown rice in Experiment 1c had no relationship with the final content of paddy in Experiment 1a and 1b. The moisture content of brown rice after first hour of soaking shown in Experiment 1c also had no relationship with moisture content of the first hour in paddy rice in Experiment 1a, but it had significant correlation with the final moisture content of paddy soaked for 24 h (Experiment 1a) and 40 h (Experiment 1b) with correlation coefficients of $r = 0.75^*$ and 0.78^{**} , respectively.

The moisture content of paddy after 1 h of soaking in Experiment 1a had significant negative correlations with time to achieve 70% germination (T_{70}) in subsequent experiments (Experiments 2, 3 and 7) but not in Experiment 4 (Table 3). Correlation coefficient with T_{70} generally decreased with increased time when moisture content was determined.

Table 3. Correlation coefficients between moisture content of paddy at different times after commencement of soaking in Experiment 1a and time required to reach 70% germination (T_{70}) in four experiments, Experiments 2–4 and 7.

Germination Time	Moisture Content at Different Times (h) after Soaking Commencement (Experiment 1a)			
	1	3	12	24
T_{70} Experiment 2	−0.62 *	−0.51	−0.41	−0.41
T_{70} Experiment 3	−0.81 **	−0.71 *	−0.47	−0.47
T_{70} Experiment 4	−0.49	−0.30	0.01	−0.36
T_{70} Experiment 7	−0.65 *	−0.71 *	−0.60	−0.43

* Significant difference at $p \leq 0.05$; ** significant difference at $p \leq 0.01$; $n = 8$.

3.1.2. Experiment 2: Germination Speed of Paddy and Brown Rice in 10 Varieties

Mean grain moisture content was significantly higher in brown rice than in paddy rice after 3 h of soaking (32 vs. 22%) and also at the time of 70% germination (38 vs. 34%) (Table 4). At 3 h, there was significant ($p < 0.001$) interaction of variety and grain type (brown, paddy). The increase in moisture content due to dehulling was greatest in YRW4 and least in Doongara. Moisture content at 70% germination varied greatly between 29 and 41% among genotypes and also there was significant interaction of variety and grain type. In both grain types, moisture content at 70% germination was the lowest in Doongara and the highest in YRW4 and Arlesienne.

Table 4. Moisture content of rice samples after 3 h soaking (MC3H) and at 70% germination (MC70%), germination rate (GR) and germination time (T_{70}) of brown rice and paddy in 10 varieties. Experiment 2.

Variety	MC3H (%)		MC70% (%)		GR (Grain/h)		T_{70} (h)	
	Brown	Paddy	Brown	Paddy	Brown	Paddy	Brown	Paddy
Doongara	24.6	20.2	25.9	31.8	3.1	2.4	29	45
Koshihikari	30.5	22.6	31.8	36.5	3	1.3	29	51
Langi	29.3	21	32.3	35	3.9	2.5	25	43
Reiziq	30.7	19.4	32.4	34.6	3.6	2.1	26	47
Sherpa	30.1	19.5	33	38.1	3.8	1.8	25	48
Tachiminori	32.4	23	35.2	39.1	3.8	2.5	24	43
YRW4	39.4	21.5	40.9	41.1	3.1	1.3	29	51
Arlesienne	33.4	26.5	37.5	42	4.6	3.9	24	36
Irat109	34.8	23.5	36.8	41	3.2	1.8	27	48
YRL24	31.5	25.1	35	39.5	4.1	3	23	39
Mean	31.7	22.2	34.1	37.9	3.6	2.3	26.1	45.1
LSD (Var)	1.1 ***		1.4 ***		0.4 ***		1.4 ***	
LSD (GT)	0.5 ***		0.6 ***		0.2 ***		0.6 ***	
LSD (Var $\times$ GT)	1.5 ***		2.0 **		0.5 ***		2.0 ***	

GT = Grain type (brown rice, paddy); Var = Variety; LSDs were obtained from ANOVA analysis; ** significant difference at $p \leq 0.01$; *** significant difference at $p < 0.001$.

There was no significant correlations between 100 grain weight and moisture content at 3 h of soaking and at 70% germination for both brown rice and paddy, with correlation coefficients below 0.38 in all cases. There were positive correlations between moisture content after 3 h of soaking and at 70% germination for both brown rice and paddy (BR $r = 0.97$ ***, paddy $r = 0.69$ *, Table 5). There was also correlation between brown rice and paddy at 70% germination ($r = 0.92$ ***), but not at 3 h of soaking.

Table 5. Correlation coefficient matrix for grain moisture content after 3 h soaking (3 h) and at 70% germination (70%) in brown rice and paddy rice. Experiment 2.

		Brown Rice		Paddy	
		3h	70%	3h	70%
Brown Rice	3 h	1			
	70%	0.97 ***	1		
Paddy	3 h	0.36	0.49	1	
	70%	0.84 **	0.92 ***	0.69 *	1

* Significant difference at $p \leq 0.05$; ** Significant difference at $p \leq 0.01$; *** Significant difference at $p \leq 0.001$.

The moisture content of paddy and brown after 3 h of soaking in this experiment had significant correlation with the moisture content of Experiment 1a for paddy ($r = 0.86$ **) and Experiment 1c for brown rice ($r = 0.89$ **), respectively.

Changes in germination level during incubation of brown rice and paddy are shown in Figure 2. Varieties varied greatly in germination level at any time particularly in paddy. The mean T_{70} from the start of soaking was 26 and 45 h for brown rice and paddy, respectively (Table 4). Germination rate of brown rice was greater than paddy by 1.3 grain/h. The interaction of genotype and grain type (brown rice, paddy) was highly significant in both germination time (T_{70}) and germination rate. The increase in germination rate and reduction in germination time (T_{70}) due to dehulling was greatest in Sherpa and least in Arlesienne and Doongara. The variety ranking in T_{70} was also different between paddy and brown rice.

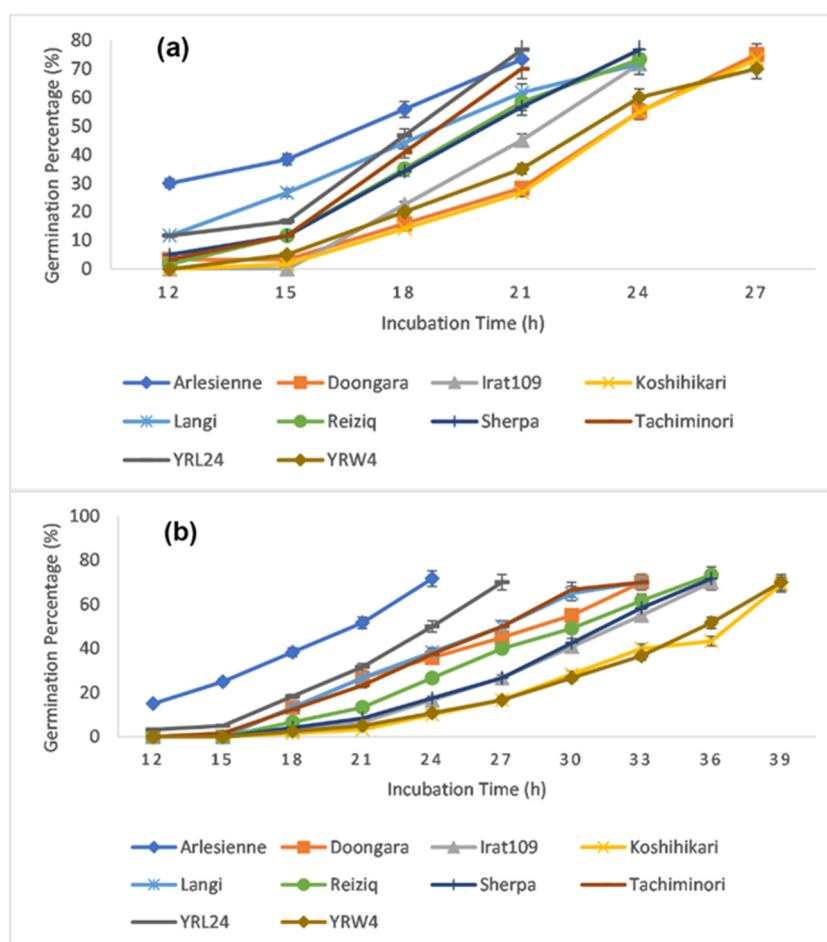


Figure 2. Changes in germination percentage of 10 varieties during incubation: (a) After 3 h of soaking in brown rice and (b) After 12 h of soaking in paddy rice. Bars indicate standard error.

There was a highly significant correlation for germination rate ($r = 0.88^{***}$) and a significant correlation for T_{70} ($r = 0.81^{**}$) between brown rice and paddy. There was no correlation between 100 grain weight and germination rate and T_{70} .

The relationship between T_{70} and grain moisture content at both 3 h of soaking and the time of 70% germination for brown rice and paddy is presented in Figure 3. The moisture content of paddy at 3 h of soaking was less than 30% in all varieties and a negative correlation with T_{70} ($r = -0.65^*$) existed. However, there was no relationship between moisture content and germination time (T_{70}) for brown rice both after 3 h of soaking and at 70% germination, and for paddy at 70% germination when most varieties had moisture content over 30%. On the other hand, the moisture content after soaking for 50 min in Experiment 1c varied from 25% to 30%, and the moisture content was significantly correlated with T_{70} of brown rice in this experiment ($r = -0.63^*$).

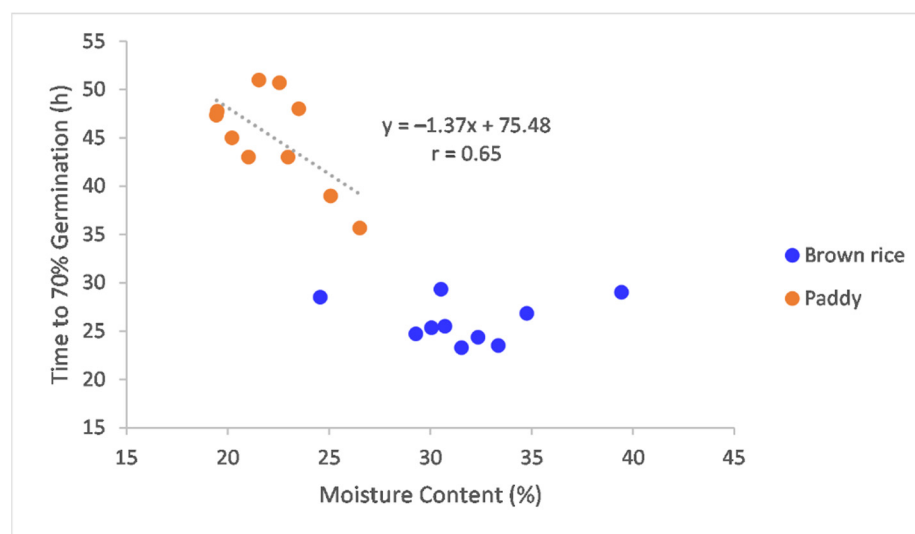


Figure 3. Relationship between moisture content after 3 h of soaking and germination time (T_{70}) in 10 varieties of brown rice and paddy. Experiment 2.

The difference in moisture content at 3 h and also in T_{70} between paddy and brown rice may be considered to be due to the hull barrier. There was a significant correlation ($r = 0.65^*$) between the difference in moisture content at 3 h and the delay in T_{70} , indicating that varieties such as Reiziq, Sherpa and YRW4 with stronger hull barrier resulted in paddies absorbing water more slowly and reaching T_{70} later (Table 4).

3.2. Consistency in Germination Speed of 8 Varieties Grown in 3 Years

3.2.1. Experiments 3 and 4: Germination of Paddy Rice in 8 Varieties in Year 1 and 2

Time to reach 70% germination from the start of soaking (T_{70}) and germination rate of paddy samples taken from well-watered and water deficit conditions in Experiment 3 from year 1 are shown in Tables 6 and 7, respectively. The mean T_{70} was 47 h, and Doongra and Langi were the quickest varieties to reach 70% germination (39 h) and TDK8 and YRW4 were the slowest (54 h). The mean germination rate was about 2 grains/h. Doongara and Langi had similar and the highest germination rate while Koshihikari, TDK8 and YRW4 had the lowest with no significant difference among them. There was a significant correlation ($r = -0.80^{**}$) between T_{70} and amylose content, indicating varieties with higher amylose content had faster germination.

Table 6. Time to achieve 70% germination (T_{70}) and germination rate (GR) of paddy rice of 8 varieties grown in well-watered (WW) conditions in Experiments 3 and 4.

Variety	Experiment 3		Experiment 4	
	T_{70} (h)	GR (Grain/h)	T_{70} (h)	GR (Grain/h)
Doonagra	39.0	3.2	36.7	2.1
Koshihikari	51.3	1.3	55.7	1.2
Langi	39.3	3.3	40.7	2.0
Reiziq	48.3	1.8	42.0	1.8
Sherpa	47.7	2.1	49.3	1.5
Tachiminori	42.3	2.5	42.7	1.7
TDK8	54.0	1.0	59.0	0.7
YRW4	54.3	1.3	51.3	1.3
Mean	47.0	2.1	47.2	1.5
LSD	2.6 ***	0.4 ***	3.2 ***	0.4 ***

*** Significant difference at $p < 0.001$.

Table 7. Time to achieve 70% germination (T_{70}) and germination rate (GR) of paddy rice of 4 varieties grown in well-watered (WW) and water deficit (WD) conditions. Experiment 3.

Variety	T_{70} (h)			GR (Grain/h)		
	WW	WD	Mean	WW	WD	Mean
Doonagra	39.0	41.3	40.2	3.2	3.0	3.1
Sherpa	47.7	51.3	49.5	2.1	1.6	1.8
Tachiminori	42.3	48.3	45.3	2.5	1.7	2.1
TDK8	54.0	54.3	54.2	1.0	1.3	1.2
Mean	45.8	48.8	47.3	2.2	1.9	2.1
LSD (Var)			1.9 ***			0.1 ***
LSD (Irri)			1.3 ***			0.1 **
LSD (Var $\times$ Irri)			2.7 *			0.2 **

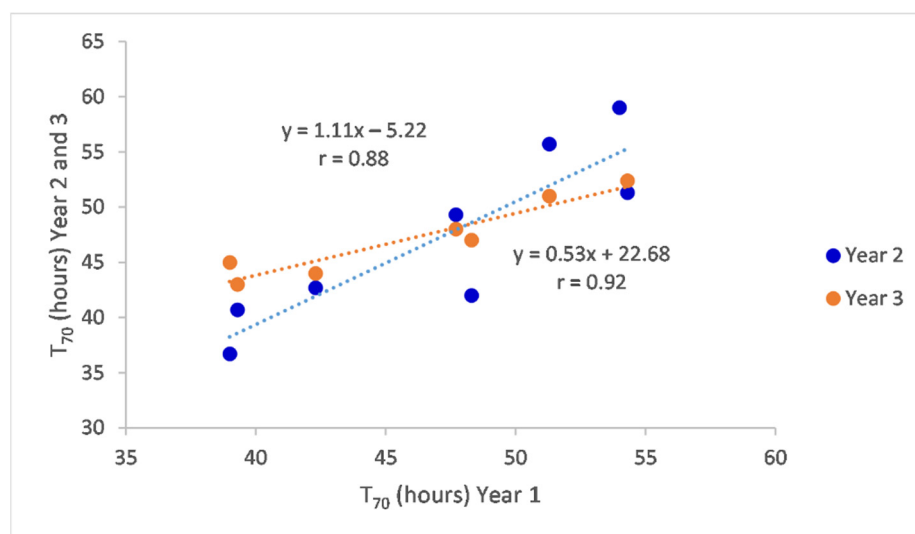
Irri = Irrigation regime (WW, WD); Var = Variety; LSDs were obtained from two-way ANOVA using split-plot design; * significant difference at $p \leq 0.05$; ** significant difference at $p \leq 0.01$; *** significant difference at $p \leq 0.001$.

The mean germination speed and germination rate of grains sampled from well-watered conditions were slightly but significantly greater than those from water deficit conditions. The interaction of variety and irrigation regime was significant in T_{70} and germination rate.

In Experiment 4, mean T_{70} was about 47 h, and Doongara was the quickest to reach 70% germination and TDK8 the slowest (Table 6). Doongara had the highest germination rate and TDK8 the lowest. The germination rate of Reiziq and Langi had no significant difference from that of Doongara while the germination time (T_{70}) of these two varieties were significantly longer than that of Doongara. When amylose content (shown in Table 1) and T_{70} (in Table 6) of these varieties was explored, a significant negative correlation ($r = -0.75$ *) existed.

3.2.2. Comparison of Germination Speed of Different Varieties across 3 Years

The results of Experiments 2 (year 3) and 4 (year 2) were compared with those obtained from Experiment 3 (year 1). All varieties in any experiments achieved T_{70} between 37 and 59 h after soaking, but the range was largest in year 2 (37–59 h) and smallest in year 3 (43–51 h). Varieties were consistent in T_{70} among 3 years' experiments (Figure 4). Correlation coefficients between any two years varied from 0.84 * to 0.92 ** for T_{70} and from 0.89 ** to 0.91 ** for germination rate.

**Figure 4.** Relationship for time required to 70% germination (T_{70}) between experiments. There were 8 varieties in year 1 and 2, and 7 varieties in year 3 experiments. Experiments 2–4.

3.3. Effect of Grain Size on Germination Speed of a Population and Diverse Varieties

3.3.1. Experiment 5: Germination of Paddy of 46 Genotypes from Sherpa/IRAT109

The mean initial grain moisture content was 13.7% with standard deviation of 0.47%. There was a significant difference in germination rate among the Sherpa/IRAT109 genotypes tested ($p < 0.001$) with mean of 3.98 grains/h (Table 8, Supplementary Table S1). The time to 70% germination (T_{70}) also varied significantly ($p < 0.001$) among genotypes with a mean of 55 h, minimum 34 h and maximum 74 h. There was a significant ($p < 0.001$) genotypic difference in final germination percentage with the average exceeding 96%. Some genotypes had ungerminated or decayed grains of up to 6 out of 50 per replication. There was no relationship between initial moisture content and germination rate or germination percentage.

Table 8. Initial moisture content (MC), 100 grain weight (GW), time to 70% germination (T_{70}), germination rate (GR) and final germination percentage (FG) of 46 Sherpa/IRAT109 genotypes. Experiment 5.

Genotype		Initial MC (%)	GW (g)	T_{70} (h)	GR (Grain/h)	FG (%)
46 genotypes from Sherpa/IRAT109	Min	12.6	1.98	34.1	2.82	89.0
	Max	14.6	3.49	74.3	5.95	100.0
	Mean	13.7	2.66	55.4	3.98	96.2
	LSD			4.2 ***	0.36 ***	4.0 ***

LSD is for 46 Sherpa/IRAT109 genotypes (see Table S1); *** significant difference at $p < 0.001$.

The 100 grain weight of Sherpa/IRAT109 genotypes had no relationship with final germination percentage but a significant negative correlation existed with germination rate ($r = -0.34^*$) and a positive correlation with T_{70} ($r = 0.40^{**}$, Figure 5a). The final germination percentage had highly significant correlations with germination rate ($r = 0.49^{***}$) and T_{70} ($r = -0.53^{***}$).

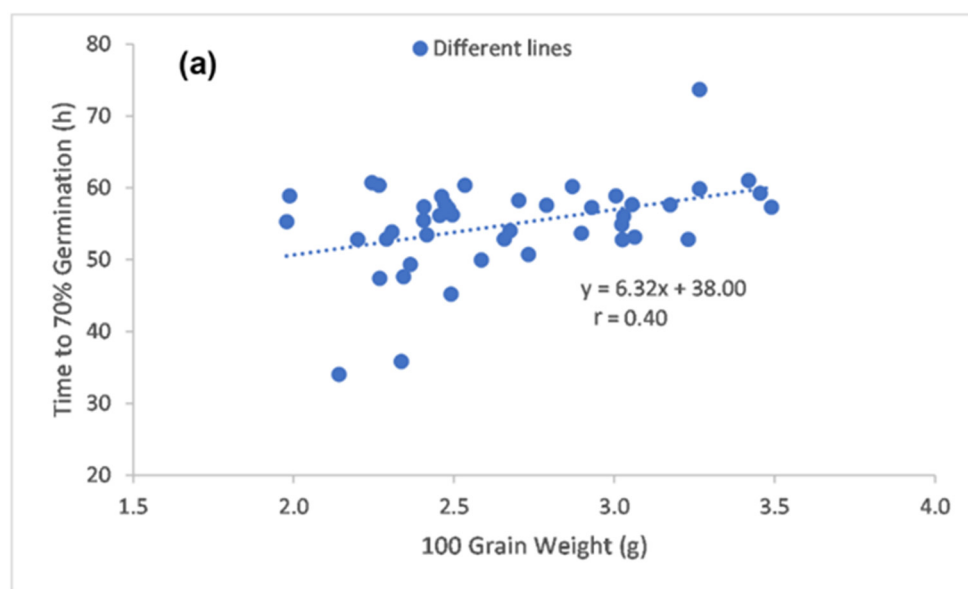


Figure 5. Cont.

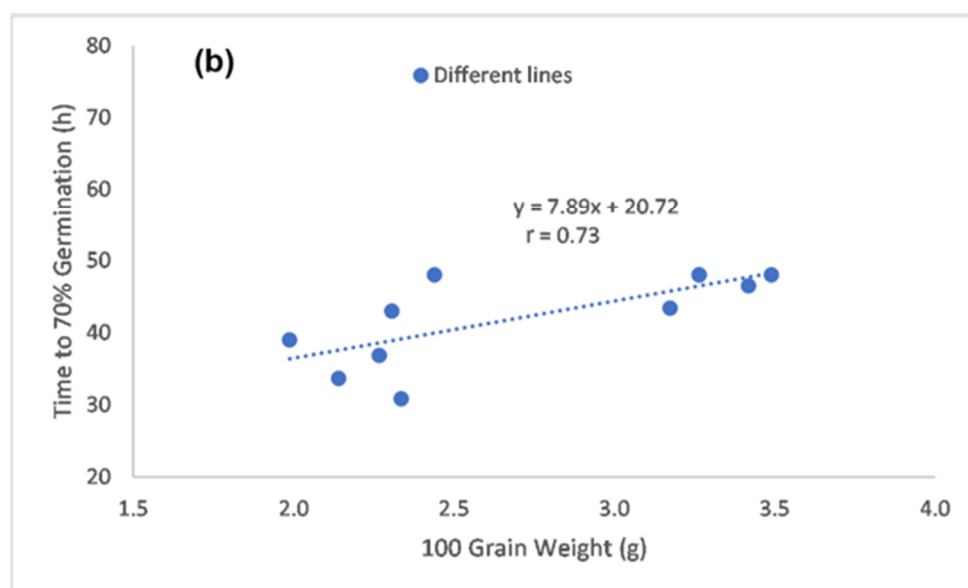


Figure 5. Relationship between 100 grain weight and time required to reach 70% germination of Sherpa/IRAT109 genotypes: (a) Experiment 5 (n = 44) and (b) Experiment 6 (n = 11).

3.3.2. Experiment 6: Germination Speed of Sherpa/IRAT109 Genotypes with Contrasting Grain Size

Among 10 genotypes examined, 100 grain weight varied from 1.99 to 3.49 g. The mean germination rate of paddy rice was 2.48 grain/h and T_{70} was 42 h with the range between 31 and 48 h (Table 9). The grains of small size germinated significantly faster than those of larger size. The mean germination time (T_{70}) and germination rate were 37 h and 3.3 grain/h for small grain genotypes and 47 h and 1.8 grain/h for larger grain genotypes, respectively.

Table 9. Initial grain moisture content (MC), 100 grain weight (GW), time to 70% germination (T_{70}) from the start of soaking and germination rate (GR) of 10 Sherpa/IRAT109 genotypes and Sherpa. Experiment 6.

Variety	Initial MC (%)	GW (g)	T_{70} (h)	GR (Grain/h)
(a) Large grain				
SHIR-602455	12.8	3.42	47	1.9
SHIR-602614	13.7	3.49	48	1.7
SHIR-602647	14.2	3.27	48	1.6
SHIR-602649	14.6	3.17	43	2.5
SHIR-603119	13.9	3.27	48	1.6
Mean	13.8	3.32	47	1.8
(b) Small grain				
SHIR-602486	12.8	2.14	34	4.1
SHIR-602517	13.2	2.27	37	3.2
SHIR-602518	13.3	2.34	31	4.2
SHIR-602557	13.7	2.31	43	2.1
SHIR-602667	13.2	1.99	39	2.8
Mean	13.2	2.21	37	3.3
(c) Sherpa	13.7	2.44	48	1.6
Mean of all entries	13.5	2.74	42	2.5
LSD			2 ***	0.4 ***

LSD is for 10 large and small grains of Sherpa/IRAT109 genotypes and Sherpa; *** significant difference at $p < 0.001$.

There was positive correlation between 100 grain weight and germination time (T_{70}) ($r = 0.73$ **) (Figure 5b), while there was negative correlation between 100 grain weight

and germination rate ($r = -0.66^*$) (Figure 6a). There was strong negative correlation ($r = -0.99^{***}$) between T_{70} and germination rate (Figure 6b).

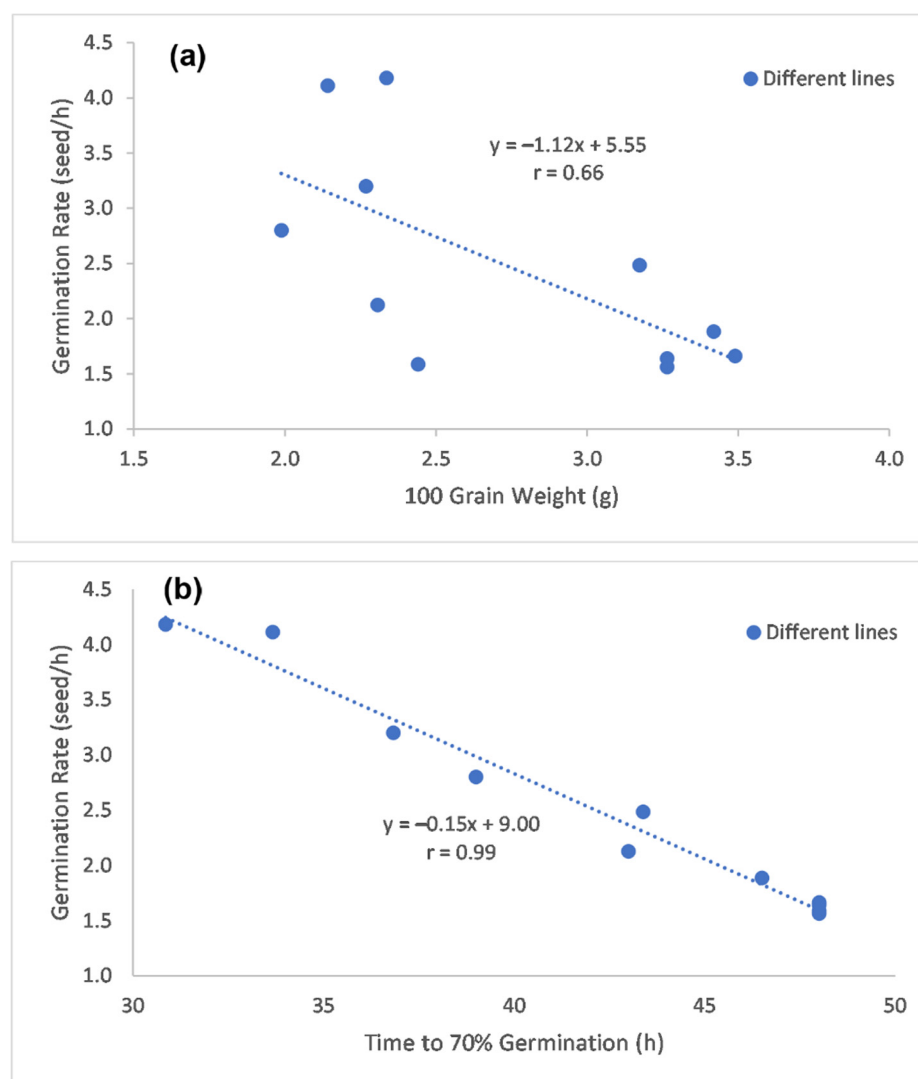


Figure 6. Relationship between germination rate and (a) 100 grain weight and (b) time to 70% germination (T_{70}) of 10 selected IRAT109-Sherpa cross genotypes and Sherpa ($n = 11$). Experiment 6.

The mean germination rate of about 2.5 grains/h was also consistent with the result of 2.7 grains/h obtained in Experiment 5. The results of germination of different genotypes were also correlated with Experiment 5, with the correlation coefficient between the experiments at $r = 0.94^{***}$ for germination rate and 0.86^{***} for T_{70} .

3.3.3. Experiment 7. Germination of Paddy of 24 Diverse Varieties

For Experiment 7, the average initial moisture content was 13.3% with standard deviation of 0.77%. There was no relationship between initial moisture content and germination rate or germination percentage.

Among 24 varieties, 100 grain weight varied from 1.44 to 3.18 g. The mean germination rate of diverse varieties was 4.03 grains/h with a minimum of 2.94 and a maximum 5.70 grains/h (Table 10, Supplementary Table S2). There was a significant difference in germination rate as well as T_{70} among all varieties examined ($p < 0.001$). The time required to reach 70% germination was about 55 h on average, with minimum of 35 h and maximum 74 h. Arlesienne had the fastest germination time of about 35 h, followed by Oscar and YRL24 at about 40 h.

Table 10. Initial moisture content (MC), 100 grain weight (GW), time to 70% germination (T_{70}), germination rate (GR) and final germination percentage (FG) of 24 diverse varieties. Experiment 7.

Variety		Initial MC (%)	GW (g)	T_{70} (h)	GR (Grain/h)	FG (%)
24 Diverse Varieties	Min	11.7	1.44	34.9	2.94	92.0
	Max	14.8	3.18	73.5	5.70	99.0
	Mean	13.3	2.37	54.6	4.03	96.5
LSD				4.9 ***	0.42 ***	3.2 *

LSD is for 24 diverse varieties (see Table S2). * significant difference at $p \leq 0.05$; *** significant difference at $p < 0.001$.

There was a highly significant genotypic difference in germination percentage with the mean exceeding 96% ($p < 0.01$). The 100 grain weight had no significant relationship with germination rate, germination time (T_{70}) or final germination percentage with correlation coefficient of 0.21^{ns}, -0.17^{ns} and 0.12^{ns}, respectively. Similarly, germination time (T_{70}) had no significant relationship with the final germination percentage ($r = -0.20^{ns}$), however, a significant negative correlation existed with germination rate ($r = -0.96^{***}$).

4. Discussion

The results of 3 years' experiments with 8 varieties show that variety differences in germination speed of paddy rice under favorable water and temperature conditions (25–30 °C) were large, but varieties were consistent with the germination speed when grown under different conditions. The range in T_{70} was 40 h among 24 diverse varieties (Experiment 7) and among the 46 Sherpa/IRAT109 genotypes (Experiment 5). While genotypic variation in germination under unfavorable conditions is well documented in relation to low temperature and water stress, for example, low temperature [23], the present work has shown large and consistent genotypic variation in germination speed under favorable germination conditions (Figure 4). Germination time (T_{70}) among varieties ranged 39–54 h in year 1, 37–59 h in year 2, and 43–51 h in year 3. Although the germination time varied over the years, there was significant correlation between germination time for any two years ($r = 0.84^*$ to 0.92^{**}). Germination percentage and speed were slightly reduced when the crop was grown under water deficit, as also found by Rahman and Ellis [28]; nevertheless, variety consistency remained in the present study. These genotypic differences in germination speed under favorable conditions are likely to have large effect in the time required for seedlings to emerge in the field, which would in turn have significant implication in early vigor and grain yield in drier conditions, particularly in early season drought in rainfed lowland ecosystem [4,6,29]. Use of varieties with early vigor would be also advantageous in competing against weeds in direct seeded rice fields [5]. On the other hand, varieties with slow germination and hence emergence would provide a greater window for application of pre-emergence herbicides [30].

With regards to the food industry, varieties with shorter processing time (soaking and incubation time) are preferred for production of germinated brown rice because shorter soaking time can result in better control of fermentation for improved final products. Furthermore, germinated grains with lower moisture content require shorter time and less energy for drying [11,31].

The large and consistent variety effect was also found in the speed of water absorption and hence grain moisture content of paddy during early period of soaking, but not at the time when 70% germination was achieved. The correlation coefficient between paddy moisture content after 3 h of soaking was $r = 0.86^{**}$ for paddies for 8 varieties between Experiments 1 and 2. This consistency in the variety differences in early water absorption has contributed to the variety consistency in the time to achieve 70% germination in paddy. The varieties with higher paddy moisture content after 3 h of soaking such as Arlesienne and YRL24 required shorter germination time (T_{70}). While the present study indicates that the first 3 h is critical, El-Hendawy et al. [12] found the significant relationship between water uptake rate during soaking and germination time in incubation period up to 48 h

of grain soaking ($r = -0.46^{***}$). Rapid water uptake may lead to a rapid activation of α -amylase which plays an important role in the degradation of starch into soluble sugars as the main substrate necessary for generating the energy required for the germination processes and subsequently for the rapid sugar mobilization from the endosperm to embryo [12,32].

Takahashi [17] reported that grains were able to fully germinate at moisture content of 25%, however, the most suitable condition for rice was 30%. The moisture content at this level was also appropriate for the production of germinated brown rice and parboiling [33]. Experiment 1 showed that the grain moisture content reached around 30% after 15 h soaking at 25 °C for paddy grains and 3 h at 30 °C for brown rice. These results were similar to the 12 and 4 h at 30 °C for paddy and brown rice, respectively, of Chai Nat 1 variety found by Rattanadee and Naivikul [33]. The results of Experiment 2 also indicated that the moisture content at 70% germination varied greatly among varieties from 29% to 41%, thus the total water required to be absorbed before a set germination percentage can be achieved would vary greatly. This would have significant consequences on processing time.

In the first 3 h of soaking in Experiment 2, brown rice of 10 varieties contained on average 8–10% higher moisture content than that of paddy. Thus, dehulling increased water uptake as the water absorption into paddy rice was initially constrained by the presence of the hull, while there was no such barrier for brown rice. Studying water absorption by paddy, brown rice and hull at three temperatures with PR116 variety, Thakur and Gupta [34] demonstrated the rigid hull enclosing the grain caused difficulty in absorbing water by paddy rice. The difference in grain moisture content continued during the germination period in the present study. This continuously higher moisture content in brown rice was also reported by Mikkelsen and Sinah [35] who found that intact grains had 2–3% less moisture content than that of dehulled grains after 35 h of soaking. They found that dehulled grains of Caloro variety germinated more uniformly than the intact grains, but the final percentage was similar to that of dehulled grains. In addition to a physical barrier, the rice hull also contained soluble substances which have an inhibitory effect on germination [35]. Kolahi et al. [36] found that rice hull extracts had allelopathic effect and could inhibit the germination of wild oat by up to 27%. In the present study, the brown rice achieved T_{70} 19 h earlier than paddy rice. Barik et al. [37] also found that germination time was decreased by dehulling particularly in semi-dormant rice varieties. The hull may act as a physical and chemical barrier to radical growth [38,39].

Experiments 1 and 2 clearly show that there were variety differences in water uptake in the early stages of soaking, but these differences also depended on grain type (paddy and brown rice) with no significant correlation for grain moisture content after 3 h of soaking between paddy and brown rice. Thus, there appeared to be significant variation in the hull barrier in water uptake in paddy, and varieties such as Sherpa which took a longer time to achieve T_{70} in paddy may be considered to have a greater barrier. The range in T_{70} was greater in paddy (36–51 h) than in brown rice (23–29 h), indicating a strong contribution of the hull barrier in determining the overall germination time.

However, there were also significant variety differences for brown rice to achieve 70% germination in Experiment 2. This was also related to moisture content in the first hour determined in Experiment 1, but not after 3 h of soaking, as moisture content of all varieties was already high, mostly exceeding 30%. Thus, rapid water uptake soon after soaking of brown rice was also a factor determining germination speed. There was a significant interaction for T_{70} between genotype and grain type (paddy and brown rice) and genotypic ranking for T_{70} also depended on the grain type. Thus, both types of barriers, one being the hull and the other the pericarp, would affect water absorption of paddy grain and subsequently influence genotypic differences in overall germination speed. The hull barrier may not be large in some cases; for example, Krishnasamy and Seshu [23] found that cultivar ranking in germination rate was the same in hulled and dehulled grains among 11 varieties. Among 68 varieties, mostly indica and semidwarf, they did not find significant correlation between hull thickness and germination rate.

The range in 100 grain weight was larger in the 24 diverse varieties (1.44–3.18 g) than in the 46 genotypes derived from Sherpa/IRAT109 RILs (1.98–3.49 g). However, the germination speed (germination rate and germination time) of diverse varieties had no significant relationship with 100 grain weight, whereas the smaller grain size Sherpa/IRAT109 genotypes tended to germinate faster than the larger grains. Smaller grains would have completed imbibition of water faster than large grains and started germinating earlier than large grains, as was shown by Roy et al. [16], Krishnasamy and Seshu [23], and Zhao et al. [40]. Krishnasamy and Seshu [23] reported that 100 grain weight of 68 rice varieties, mostly semidwarf indica varieties, had a negative correlation with imbibitional rate and also germination rate. Zhao et al. [40] compared superior grains obtained from primary branch of a panicle and inferior grains from secondary branch for the germination vigor of dehulled grains of Xinfeng 2. Superior grains had double the weight of inferior grains and it also had better structured starch compared to looser structure in the inferior grains. The inferior grains absorbed water 1.6 times faster within 6 h and had greater germination vigor in the first 24 h of soaking. Bhawana et al. [41] found that grain weight and germination percentage (together with shoot dry weight and shoot length) were the characteristics mainly responsible for differences in germination speed among 50 aromatic rice varieties. Similarly, in the present study, a significant effect of 100 grain weight was found in Sherpa/IRAT109 genotypes, but this was not the case in the 24 diverse varieties. This may be because of the strong kinship among the RILs, as a result of genotypes emanating from a single cross (Sherpa/IRAT109), and hence the effect of 100 grain weight was detected. In contrast, the genetic background of the 24 diverse varieties differed greatly. As shown in Experiment 2, there were barriers to grain moisture absorption, and they may have masked the effect of grain size.

5. Conclusions

The present study demonstrated that significant variation existed among rice varieties in terms of germination speed. The ease of water uptake and the grain size were the main factors contributing to this variation. Water uptake of paddy rice in the early stage of soaking had a significant relationship with germination speed, and varieties with higher grain moisture content after 3 h of soaking tended to germinate faster. Water uptake in paddy was constrained by the hull since it acted as a barrier to water uptake and subsequent germination for paddy rice. Grain moisture content after 3 h of soaking in paddy was 10% less than in brown rice, and paddy rice achieved 70% germination 19 h later than brown rice. Among genotypes derived from a single cross Sherpa/IRAT109, those with a smaller grain size germinated faster than genotypes with larger grain size. However, this relationship was not evident among 24 varieties mostly from a japonica diversity set, confirming suggestions in the literature that within a variety, grain size affected germination speed. The existence of genotypic variation in germination speed has management implications for both field crop establishment and paddy germination in food processing.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11081614/s1>, Table S1: Initial moisture content (MC), 100 grain weight (GW), time to 70% germination (T70), germination rate (GR) and final germination percentage (FG) of 46 Sherpa/IRAT109 lines. Experiment 5, Table S2: Initial moisture content (MC), 100 grain weight (GW), time to 70% germination (T70), germination rate (GR) and final germination percentage (FG) of 24 diverse varieties. Experiment 7.

Author Contributions: Conceptualization, S.C. and S.F.; methodology, S.C.; validation, S.F. and J.M.; formal analysis, S.C.; investigation, S.C.; resources, J.M.; data curation, S.C.; writing—original draft preparation, S.C.; writing—review and editing, S.F. and J.M. All authors have read and agreed to the published version of the manuscript.

Funding: Sinh Chao gratefully acknowledges the financial support of the Australian Centre for International Agricultural Research (ACIAR) through the John Allright Fellowship. Operational support was partially funded through ACIAR project CSE-2012-077, and partially funded by AgriFutures project PRJ-011067.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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