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Establishment rate of direct-seeded rice in the relay-intercropping system in Kanto region of Japan

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ABSTRACT

The relay intercropping system for cultivating direct-seeding rice and winter cereal is a low-cost method particularly for double cropping, because it eliminates the steps of raising seedlings, paddling, and transplanting. However, in this system, the seedling establishment rate (SER) of rice is low and unstable. The objective of this study was to identify the factors affecting SER to highlight the ways to improve SER. Experiments were conducted in experimental fields in Tsukuba City, Ibaraki Prefecture in 2015 and 2016. To determine the time of rice seed death and to calculate seed survival rates, 'Akidawara' seeds were embedded in soil (depth: 3 cm) and dug up after defined periods. We then analyzed the correlation between SER and meteorological factors at two sites (Ibaraki Prefecture and Gunma Prefecture) over the same two years. Based on mean air temperature (MT), we divided the period from seeding to June 20 (when irrigation had been initiated in almost all the fields) into four phases. In Phase 1, the number of days with rainfall (\geq 5 mm) and soil-wetting days (water potential \geq -100 kPa) were significantly and negatively correlated with SER. We found that most of the seed deaths occurred after germination, and the germination rates were presumably affected by water absorption during the low temperature phase. Further investigations are needed to understand the occurrences during the seedling emergence period. This study contributes to a better understanding of the factors affecting variations in the SER of direct-seeded rice grown in the relay-intercropping system.

List of Abbreviations: DSR: direct-seeded rice; SER: seedling establishment rate; TT: thiuram treatment; SSR: seed survival rate; DS: dead seeds; AGS: already germinated seeds; DSRI: direct-seeding of rice in the inter-row spaces of winter cereals; VWC: volume water content; WP: water potential

Introduction

In Japan, double cropping covers an area of approximately 83,000 ha (Ministry of Agriculture, Japan, 2015), of which 14,200 ha is within the Kanto region. Rice is generally planted from April to May in this region. In the double-cropping areas, however, winter cereals are not harvested until early June, and rice is planted in the mid- or late June after tillage and irrigation of the field. In double-cropping systems, the workload (i.e. harvesting of winter cereals, tillage, plowing, and transplanting) in June is severe for large-scale farmers. Raising and transplanting seedlings occupy almost one-quarter of the working hours during rice cultivation (Ministry of Agriculture, Japan, 2016). Direct seeding of rice (DSR) in the wet or dry paddy fields is an alternative practice that eliminates the necessity of raising seedlings and transplanting them, and this practice is increasingly attracting the attention of researchers and farmers. In the double-cropping area of the Kanto region, however, ARTICLE HISTORY

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KEYWORDS

Intercropping system; direct-seeded rice; seedling establishment; winter cereals

the practice of DSR has not increased, because of the unstableness of the yield resulting from late sowing. Relay intercropping of direct-seeded rice and winter cereal might distribute the workload and resolve the problem by ensuring a longer growing period because of early seeding. In this system, rice seeds are directly seeded into the inter-rows of winter cereals (e.g. wheat or barley) in March or April. During the harvest of winter cereals, rice seedlings usually grow to about 10 cm in height, which helps the farmers start cultivating the rice immediately after harvesting the winter cereals, just by starting irrigation. We have previously reported (Maki, Hosobuchi, Kojima, Yasumoto, & Ohshita, 2016) that the yield of the direct-seeded rice grown in the inter-row spaces of winter cereals (DSRI) is possibly more stable than that of the DSR grown after harvesting winter cereals, and that the yield of DSRI rice can be higher depending on the year. Thus, DSRI is possible to reduce and distribute the workload as well as provide stable yield. However, despite some of its

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constituent techniques being well established, such as the seed thiram treatment (TT) (Maki et al., 2016), the DSRI technique is still being developed and the seedling establishment rate (SER) of DSRI rice can be improved to ensure higher and more stable yields. This lacuna is suspected to be one of main reasons preventing the wide-spread application of DSRI in the Kanto region, which only uses 10 ha for DSRI. To improve SER, it is necessary to identify the causes for the low and unstable SER in DSRI.

There are many reports about seedling establishment in DSR, but little is reported on the SER in DSRI. The SER in DSRI is lower than that in bare fields (Hamana & Kobayashi, 1978). These authors reported that SER was affected by the distance from the wheat row and improved by TT in the Tohoku region. Very early DSRI in autumn or winter were challenged by Himeda and Fujii (1969), who reported that early seeding reduced SER. These authors also emphasized that physiological phases of many seeds proceed under comparatively higher temperatures, following which the plants decay with subsequently decreasing temperatures (i.e. in January). However, further research is needed because winter cereal cultivation (i.e. inter-row spacing) methods have now changed. Furthermore, these studies were conducted only on experimental fields, and the effects of meteorological factors and the differences between actual farming sites were not considered. Therefore, information on the factors inhibiting yields in DSRI is limited.

In the present study, we investigated the time of seed death under DSRI through seed-embedding experiments. Additionally, to identify the factors affecting SER, we analyzed the correlations between seedling mortality and meteorological factors, and the effect of soil types in the experimental fields. Based on the SERs detected in the study sites, we have discussed the complex underlying factors that affect SER and suggested potential approaches to improve it.

Materials and methods

1. Seed-embedding experiment

1.1 Study sites

The two experimental fields of the National Organization of Agriculture and Food Research Organization (NARO) are located in Tsukuba City, Ibaraki Prefecture (36°1'N, 140°5'E, altitude: 22.7 m).

1.2 Plant material

Oryza sativa L. 'Akidawara' seeds that had been collected from the previous year were used for cultivation

in the experimental fields. Germination rates of the seeds were >95% after incubation at 30°C for 7 d.

1.3 Experimental design

To evaluate the effect of TT, we embedded rice seeds packed in mesh bags, with or without TT (40% tetramethylthiuram disulfide (TMTD), Kihigen R-2 Flowable, Yonezawa Chemical Corporation, Kyoto Prefecture, Japan) in the gray lowland soil of the experimental fields. Each mesh bag contained 30 seeds. The embedding dates were 12 March 2015 and 2 March 2016. Every one or two weeks, the bags were dug up to investigate the survival rates of the seeds. This experiment included three replications (mesh bags) per treatment (TT, and non-treated i.e. NT). The collected seeds were washed and used in the following analyses.

In 2016, we counted the seeds that had already germinated (AGS, already germinated seeds) at the time of being dug up, based on the modified method of Ohdaira and Sasaki (2011). The AGS included seeds that showed indications of germination, such as the presence of a shoot or a trace of germination. The seed survival rate (SSR), which refers to the proportion of live seeds, was calculated using the following two methods: (i) counting the germinated seeds after incubation at 30°C for 10 d; and (ii) the squash method (Baskin & Baskin, 1998) and modified staining method using triphenyl tetrazolium chloride (TTC) (Porter, Durrell, & Romm, 1947) to test the survival of the non-germinated seeds. Dead seeds (DS) refer to the seeds that were determined to no longer be alive after being subjected to methods (i) and (ii). However, seeds that had been damaged at the time of digging up could not be convincingly categorized as AGS and were counted as DS. Thus, DS estimates may include AGS that had germinated early during the experiment.

To investigate the effect of soil types, the seeds were embedded in the bare spaces of an andosol field and a gray lowland-soil field in 2015. To investigate the effect of winter crops, rice seeds were embedded in the bare spaces and inter-rows of wheat in the gray lowland-soil field in 2016. Soil moisture probes (EC-5, Decagon Devices, Pullman, WA, USA) and water potential probes (MPS-6, Decagon Devices, Pullman, WA, USA) were connected to data loggers (Em50, Decagon Devices, Pullman, WA, USA) to constantly monitor the soil volume water content (VWC), soil temperature, and soil water potential (WP), throughout 2016. Sensors were inserted into the ground at 5 cm depth for WP and 3 cm depth for VWC, in the bare spaces and interrow spaces of winter cereals.

2. Relationship between SER and the meteorological factors and cultivation conditions

2.1 Study sites

We conducted the following experiments at two sites in the Kanto region of Japan, as listed in Table 1. One site, hereafter referred to as Site 1, was located in Tsukuba City (as described in section 1.1), and experiments were conducted in the experimental fields of NARO. The other site, hereafter referred to as Site 2, was located in Sibukawa City, Gunma Prefecture (36°28'N, 138°59'E, altitude: 182.8 m), and experiments were conducted in the fields belonging to farmers.

2.2 Plant material

Oryza sativa L. 'Akidawara' and 'Hoshijirushi' were used for cultivation in Site 1, and 'Yumeaoba' was used for cultivation in Site 2. All seeds used for cultivation in Site 2 were prepared by the farmers.

2.3 Determination of SER and cultivation conditions

To identify the causes of SER alterations, we collected data on SER and cultivation conditions (i.e. seeding date, types of seeders, winter cereals, and soils) from the two sites (Table 1). In 2012 and 2013, SER was investigated in one field at Site 1. In 2015 and 2016, SER was investigated in two fields at Site 1 and Site 2. All the seeds used for DSRI were subjected to TT (using 40% TMTD). DSRI was conducted via the following two methods: (i) Early seeding of rice in six

Table 1. Sites and rice cultivations.

rows using a non-tillage seeder (NSV600, Matsuyama Co., Ueda City, Japan), with an inter-row space of 30 cm. Because in this system tractors operate in the fields during the growth of winter cereals, rice seeding must be conducted before stem elongation of the winter cereals. (ii) Late seeding of rice in three double rows using a highclearance type seeder (Intercropping seeder, Sasaki Co., Towada City, Japan), with an inter-row space of 30–40 cm. In this method, seeding can be conducted even after the heading of winter cereals. In Site 1, the seeding rates were $8-9 \text{ g m}^{-2}$. SER was counted just after the harvest of the winter cereals. The area surveyed for determining SER was between 1.2 and 2.4 m² per plot. In Site 2, SER was counted in three 1.6–2.4 m² areas per field, just before or after the harvest of winter cereals. Although we counted the SER excluding the seedlings derived from dropped seed of rice cultivated in the previous year, they may have slightly affected SER estimates, especially in Site 2.

2.3 Collection of meteorological factors

Meteorological data (mean temperature (MT) and rainfall) of the nearest observation point were collected from the Automated Meteorological Data Acquisition System (AMeDAS) (http://www.data.jma.go.jp/obd/stats/etrn/index.php). For Site 2, the average value of the two nearest observation points was adopted. From the MT of five days before and after the day determined, we divided the period from seeding to June 20 into four phases. June 20 was set as the starting date of irrigation in this relay intercropping system. We defined each phase as follows: Phase 1: MT \leq 10°C, Phase 2: 10°

Site							Date of	Type of winter
name	Site location	Year	Field	Soil type	Cultivers	Type of seeder	seeding	cereal
Site 1	Ibaraki Pref. Tsukuba	2012	Field1	Andosol	Hoshijirushi	Non-tillage seeder	16-03-2012	Barley
			Field1	Andosol	Hoshijirushi	Intercropping seeder	16-03-2012	Barley
		2013	Field1	Andosol	Hoshijirushi	Non-tillage seeder	19-03-2013	Barley
			Field1	Andosol	Hoshijirushi	Intercropping seeder	19-03-2013	Barley
		2015	Field2	Gray lowland soil	Akidawara	Non-tillage seeder	13-03-2015	Barley
			Field1	Andosol	Akidawara	Non-tillage seeder	13-03-2015	Barley
			Field2	Gray lowland soil	Akidawara	Intercropping seeder	02-04-2015	Barley
			Field1	Andosol	Akidawara	Intercropping seeder	02-04-2015	Barley
			Field2	Gray lowland soil	Akidawara	Non-tillage seeder	13-03-2015	Wheat
			Field1	Andosol	Akidawara	Non-tillage seeder	13-03-2015	Wheat
			Field2	Gray lowland soil	Akidawara	Intercropping seeder	02-04-2015	Wheat
			Field1	Andosol	Akidawara	Intercropping seeder	02-04-2015	Wheat
		2016	Field2	Gray lowland soil	Akidawara	Non-tillage seeder	26-02-2016	Barley
			Field1	Andosol	Akidawara	Non-tillage seeder	26-02-2016	Barley
			Field2	Gray lowland soil	Akidawara	Non-tillage seeder	26-02-2016	Wheat
			Field1	Andosol	Akidawara	Non-tillage seeder	26-02-2016	Wheat
			Field2	Gray lowland soil	Akidawara	Non-tillage seeder	26-02-2016	Wheat
			Field1	Andosol	Akidawara	Non-tillage seeder	26-02-2016	Wheat
			Field2	Gray lowland soil	Akidawara	Non-tillage seeder	26-02-2016	Wheat
			Field1	Andosol	Akidawara	Non-tillage seeder	26-02-2016	Wheat
Site 2	Gunma Pref.	2015	Field3	Skeletal gray lowland soil	Yumeaoba	Non-tillage seeder	06-03-2015	Wheat
	Shibukawa		Field4	Skeletal gray lowland soil	Yumeaoba	Non-tillage seeder	05-03-2015	Wheat
		2016	Field15	Skeletal gray lowland soil	Yumeaoba	Non-tillage seeder	25-02-2016	Wheat
			Field16	Skeletal gray lowland soil	Yumeaoba	Non-tillage seeder	25-02-2016	Wheat

 $C < MT \le 15^{\circ}C$, Phase 3: $15^{\circ}C < MT \le 20^{\circ}C$, and Phase 4: MT > 20°C. We defined the phases as irreversible. If there were five consecutive days of next phase, the first day of this five-day period was regarded as the day when the next phase had started.

WP was recorded in one field in each site, using soil moisture probes, in Phases 1–3, as described above. WP probes were MPS6 in 2016 in both sites. In 2015, MPS1 and MPS2 were used in Site 1 and Site 2, respectively. Soil-wetting days were the number of days when the recorded WP was above -100 kPa.

3. Statistical analysis

Statistical analysis was performed using JMP v. 11.0 software (SAS, Inc. Cary, North Carolina, USA). Statistically significant differences between mean SER of soil types, types of seeder, and types of winter cereals were evaluated by analysis of variance (ANOVA). For soil type factor analysis, we used the SER data of DSRI, which used a non-tillage seeder for 2015 and 2016. For type of seeder factor analysis, we used the SER data for 2012 and 2013, and for type of winter cereals analysis, we used the SER data of DSRI (non-tillage seeder) in the andosol field for 2015 and 2016. Correlation analyses were conducted on the SER data of 2015 and 2016 of Site 1 and Site 2, both of which were only seeded by the non-tillage seeder.

Results

1. Seed embedding experiment

The increase of AGS started slower in the inter-row spaces than in the bare spaces in 2016, regardless of TT (Figure 1). From April 2 to May 9, AGS in the bare spaces was significantly higher than that in the interrow spaces (P < 0.05). In 2015 and 2016, the seeds subjected to TT had higher survival rates than the NT seeds (Figure 2). The SSR of the NT seeds started to decrease earlier than that of the TT seeds. In the bare spaces, significant differences of SSR between TT and NT began around April 9 in 2015, and around March 17 in 2016. The dynamics of SSR in the inter-row spaces was like that in the bare spaces, but significant differences in SSR dynamics between these spaces occurred on April 22. In 2015, the SSR was slightly lower in the gray lowland soil than in the andosol at the end of April (Figure 3). VWC and WP between the winter cereal rows were lower than those in the bare spaces (Figure 4). The diurnal variation in soil temperatures in the inter-rows was less than that in the bare spaces (Figure 5), and the



Figure 1. Transition of the rates of the already germinated seeds (AGS) and dead seeds (DS) in 2016. Error bars indicate standard deviations. Denominator of each rate indicates the number of seeds dug up.

mean soil temperature was lower than that in the bare spaces.

2. Effect of cultivation conditions on SER in Site 1

The results of ANOVA are shown in Table 2. SER in the fields with andosol (20.73%) were significantly higher than those in the fields with gray lowland soil (7.53%) (P < 0.01). There were no significant differences in SER with respect to the type of seeder used and the type of winter cereal cultivated.

3. Relationship between SER and meteorological factors

In Phase 1, there was a significant negative correlation between the number of rainfall days (≥ 5 mm) and SER (r = -0.505, P < 0.05, Table 3). Moreover,



Figure 2. Change in the seed survival rates in 2015 and 2016 in gray lowland-soil field. Error bars indicate standard deviations.

NS, not significant (P > 0.05)

** and * indicate significant differences at 1% and 5% levels, respectively.

Data for 30 April 2015 could not be evaluated by ANOVA, because the STDEV. was 0.



Figure 3. Changes in the seed survival rates in the different soil-type fields (2015). Error bars indicate standard deviations.NS, not significant (P > 0.05)** and * indicate significant differences at 1% and 5% levels, respectively.Data for 30 April 2015 could not be evaluated by ANOVA, because the STDEV. was 0.



Figure 4. Volume water content (VWC, line graph) and water potential (WP, bar graph) in 2016.



Figure 5. Soil temperature in 2016.

Table 2. Mean seedling establishment rate (SER) and the effects of fixed factors in Site 1

Fixed Factor		Mean SER (%)	P-value
Soil type	Andosol	20.73	0.001**
	Gray lowland soil	7.53	
Type of seeder	Non-tillage	23.85	0.587
	Intercropping	24.73	
Type of winter cereal	Barley	15.13	0.631
	Wheat	13.63	

There were no significant effects of interactions among factors.

** and * indicate significant differences at 1% and 5% levels, respectively.

soil-wetting days (The number of days the recorded WP was above -100 kPa) were significantly and was negatively correlated with SER (r = -0.606, P < 0.05, Table 3, Figure 6A). The number of days (the duration of Phase 1) was positively correlated with SER (r = 0.642, P < 0.01, Table 3, Figure 6B). The total number of days (Phases 1–4, the duration from seeding to the end of Phase 4) had significant positive correlations with SER (r = 0.524, P < 0.05,

Table 3). There were no other factors correlated with SER in Phases 2–4.

Discussion

1. Underground dynamics of seed state

In TT seeds, AGS rates reached about 40% by April 1. AGS + DS rates of TT seeds at the end of April were almost higher than 90% (Figure 1), and the SSR were over 60% by May 2 (Figure 2). These findings indicate that almost all rice seeds started germinating at least one month prior to the harvest of winter cereals (early June), and that the majority of rice seeds were still alive at that time. However, SER in the same field was much lower than SSR (Table 2), suggesting that seed mortality occurred almost entirely between May and June. These findings indicate that most of the seeds did germinate, but could not emerge from the soil surface, or probably died after emergence, owing

Table 3. Relationship of seedling establishment rate (SER) and meteorological factors.

	<u> </u>	2		
Phase	Meteorological factor	Correlation coefficient	P-value	Significant difference
1–4	Total number of days (Phases 1–4)	0.524	0.037	*
$1 (MT \le 10^{\circ}C)$	Number of days	0.642	0.007	**
	Mean temperature	-0.310	0.243	NS
	Total rain fall	-0.408	0.117	NS
	Number of rain falls (\geq 5 mm)	-0.505	0.046	*
	Number of soil wetting days (WP \geq -100)	-0.606	0.013	*
2 (10°C < MT ≤ 15°C)	Number of days	0.168	0.535	NS
	Mean temperature	0.303	0.254	NS
	Total rain fall	-0.103	0.705	NS
	Number of rain falls (\geq 5 mm)	0.329	0.213	NS
	Number of soil wetting days (WP \geq -100)	-0.249	0.352	NS
3 (15°C < MT \leq 20°C)	Number of days	-0.275	0.303	NS
	Mean temperature	0.145	0.592	NS
	Total rain fall	-0.316	0.233	NS
	Number of rain falls (\geq 5mm)	-0.323	0.222	NS
	Number of soil wetting days (WP \geq -100)	-0.441	0.087	NS
4 (MT > 20°C)	Number of days	0.128	0.637	NS
	Mean temperature	0.118	0.664	NS
	Total rain fall	0.032	0.907	NS
	Number of rain falls (\geq 5 mm)	0.101	0.711	NS

NS, not significant (P > 0.05)

** and * indicate significant differences at 1% and 5% levels, respectively.



Figure 6. Correlation plot of seedling establishment rate (SER) and meteorological factors in Phase 1. (A) Soil-wetting days (the number of days that the water potential was above -100 kPa). (B) Number of days (the duration of Phase 1).

to other stressors. One possible explanation is that physiological germination under low temperature delayed shoot elongation; and seed vigor was consequently exhausted before emergence. This is supported by the findings of a previous study (Himeda & Fujii, 1969) that emphasized the harmful effects of low temperature on physiological processes following seed germination, and also by the findings of by Furuhata, Iwaki, and Arima (2006), wherein they suggested that delayed emergence of the rice seedlings increases seedling mortality in DSR. The analysis of meteorological factors in our study also supports this hypothesis. The number of days with rainfall (≥5 mm) and soil-wetting days in Phase 1 were significantly and negatively correlated with SER (Table 3). Phase 1 was characterized by temperatures that were generally below the lower thermal limit of germination. Although some of the seeds are able to germinate under these conditions, germination is slow. In addition, the results of the seed-embedding experiment indicated that SSR in 2015 (less rainfall in Phase 1) was higher than that in 2016, indicating that dry conditions are required to maintain seed vigor, if DSRI is conducted in low-temperature conditions.

However, drought also seems to affect SER. Hamana and Kobayashi (1978) emphasized that SER in DSRI, which were seeded near the row of winter cereals, was low. lijima, Asai, Zegada-Lizarazu, Nakajima, and Hamada (2005) suggested that rice seedlings and wheat might compete for water in a sequential double-cropping system with no-tillage seeding of wheat. Therefore, appropriate soil-water conditions are needed to ensure the stability of SER in DSRI.

2. Effects of TT, soil types, seeder types, and other factors

We found that TT increased the survival rates of the embedded seeds, irrespective of whether they were planted in bare spaces or inter-rows (Figure 2). TT can improve SER in DSR by preventing infection from pathogenic bacteria (Shu, 1987). Hamana and Kobayashi (1978) reported that TT improved SER in the inter-rows of wheat, which is consistent with our findings.

SSR and SER were significantly higher in the andosol field than in gray lowland soil (Figure 3 and Table 2). Andosol shows high permeability when used for rice cropping (Ebina et al., 1990). Our findings indicate that the physical properties of soil may affect SER.

Another possible factor is seed dormancy. High-dormancy varieties have high overwintering abilities (Ohdaira & Sasaki, 2011). There may also be some allelopathic effects of winter cereals on DSRI.

3. Methods of improving SER

We found that some environmental factors affect the SER in DSRI, such as rainfall in Phase 1 (i.e. water absorption at low temperature conditions) and soil types. Seed treatment and appropriate irrigation may be effective in improving SER in DSRI. In addition, some other methods have been suggested, such as multilayer treatment with thiram (Hamana & Kobayashi, 1978), and paddling and leveling in autumn (Nakajima, Hamada, Ikeda, & Shaku, 2006). However, these treatments do not offer a holistic solution as different sites have different influencing factors. We investigated the effect of applying more thiram; although a slight effect was observed in the andosol field, no effect was observed in the gray lowland-soil field (data not shown). The method of paddling and leveling in autumn cannot be easily adopted, because in most paddy-cultivating areas in Japan irrigation is limited only to the rice cultivation period (spring-summer). The present findings indicate that early sowing may affect SER negatively because of rainfall in Phase 1; therefore, treatment aimed at prevention of water absorption and selection of varieties that show enduring seed vigor even in the face of unsuitable temperature must be researched in future.

Conclusion

We investigated when and why DSRI seeds die. TT significantly increased SSR; however, despite being subjected to Π , the majority of the seeds died after germination in May. SER was significantly correlated with total rainfall in Phase 1 (from the seeding date to about the end of March). These findings and previous reports suggest that water absorption during low-temperature periods may decrease seed vigor and increase seed mortality following germination. Our findings suggest that early initiation of DSRI causes a decrease in SER owing to exposure to rainfall in Phase 1. To improve SER in early DSRI (late February and March), it is necessary to consider seed treatments that prevent water absorption during Phase 1. Further investigations are needed to clarify the factors affecting seed death after germination in May and June.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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