Establishment of Rice Seedlings by Direct Sowing of Multiple Seed Pellets on Paddy Soil Covered with Legume Living Mulch

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Recently, low input and high sustainability in agricultural production have received much attention. Cultivation systems with cover crops have been studied (Ueno, 2004). The cultivation methods with cover crops offer several advantages that include biological nitrogen fixation, labor savings associated with minimum tillage, and physical and chemical weed suppression (Fujii, 1995; Fujihara and Yoshida, 2000). In the rice cultivation systems, rice seedlings are transplanted or the seeds are sown directly. Direct sowing of rice saves labor and production cost. However it is difficult to control weeds in this system, application of herbicide tends to increase compared with rice transplanting (Tomihisa, 1993; Watanabe and Kawana, 2006). The combination of the direct sowing method and cover crop may be effective for weed control, and improve low input and sustainable rice cultivation system, if methods that assure uniform seedling establishment and stable yields are established.

Nakano (1999) and Nakano and Sugimoto (1999) conducted no-tillage rice cultivation experiments by furrow sowing in a well-drained paddy field covered with legume mulch. They reported that low light intensity under the canopy of some green manures, 5%-10% daylight, resulted in a reduced soil temperature and a low rate of rice seedling establishment. Moreover, approximately 20% daylight under the canopy of the other green manures resulted in the same rate of establishment as that on bare ground. However, little is known about the rates of seedling establishment in broadcast sowing on the soil surface covered with legume mulch. Asagi et al. (2004) reported that the rate of seedling establishment was very low (2.7%) in broadcast sowing on the soil surface covered with legume mulch. It may be because the contact between seeds and soil is difficult to control in broadcast sowing on a well-drained paddy field covered with legume mulch. Therefore, it is necessary to provide appropriate cultivation method for better seedling establishment.

Seed coating with clay may improve moisture retention and facilitate seed emergence and seedling establishment in broadcast seeding. As a new technology for rice direct seeding, multiple seed pellets (MSP)—presoaked rice seeds coated with clay and shaped into spheres—have been developed at the National Agricultural Research Center for Tohoku Region, Japan with the objective of improving lodging resistance and consistency in seedling establishment. Sowing of MSP has now become a practical method for direct seeding in flooded paddy fields (Yaji et al., 2001). If the use of MSP would improve the rate of seedling establishment in broadcast sowing on the soil surface covered with legume mulch, this direct sowing system would overcome the difficulty of controlling weeds, and would be a useful system for low input and high sustainability. However, direct sowing using MSP on a well-drained paddy field covered with legume mulch has not been almost examined. Therefore, using MSP, we conducted a preliminary research on the effects of seed coating with clay on seedling establishment in rice paddy soil covered with living legume mulch.

Materials and methods

1. Site conditions

Experiments were conducted at the University Farm, Ehime University, Matsuyama City, Japan. The mean ambient temperature during the cultivation period (June 9-25, 2005) was 24.4°C, and this was approximately 1.9°C higher than the average for the
30-year period between 1971 and 2000.

2. Preparation of rice seed

Rice seed (*Oryza sativa* L. cv. “Matsuyamamii”) with a specific gravity greater than 1.15 was collected for use in this study. The dry seed were incubated with chemical fungicide solution at 20°C for 1 day, and the presoaked seeds, just before sprouting, were prepared by soaking the dry seeds in water at 20°C for 4 days previously. After treatment with chemical fungicide, a portion of the presoaked seeds was coated with clay (dry weight, clay : seed = 2 : 1) or with diatomaceous clay (dry weight, 15% diatomaceous clay : seed = 2 : 1) to improve moisture retention. Rice seeds were coated without CaO₂ for oxygen supplement because oxygen was considered to be enough for seed emergence and seedling establishment in broadcast sowing on the soil surface.

MSP was prepared by Automatic Granulation System of Multiple Seed Pellet (Sekiya et al. 2004), which pelletize presoaked seeds with clay or diatomaceous clay by the rotary motion of 2 wet sponge sheets. Each of the pellets—coated with either clay or diatomaceous clay—consisted of an average of 9 presoaked rice grains and exhibited the following chemical properties: pH (pellet : water = 1 : 5), 6.28 and 6.31; electrical conductivity (EC), 9.08 and 9.37 μS m⁻¹ for clay and diatomaceous clay pellets, respectively. There were no significant differences in pH and EC between clay and diatomaceous clay pellets (t-test, p=0.05, n=4).

3. Seed germination in the incubation test

In order to evaluate the potential rate of seed germination, 20 dry seeds, 20 presoaked seeds and 5 MSP with either clay or diatomaceous clay were incubated at 22°C in petri dishes containing 10 ml of distilled water. The number of germinated seeds (seedling length >1 mm) was measured at 3 and 5 days after sowing (DAS) with 4 replications.

4. Direct sowing in pots with living mulch

As a legume, white clover (*Trifolium repens* L.) was sown in Wagner pots (area, 0.02 m²; height, 20 cm), each filled with approximately 3 kg of dry soil on April 19, 2004, and cultivated under field conditions. The soil was collected from the paddy field at the University Farm, Ehime University and had the following properties: 1.46% total carbon (TC) and 0.15% total nitrogen (TN); it comprised of 15.2% clay, 10.7% silt, and 74.0% sand.

On June 9, 2005, the 4 types of rice seed (dry seeds, presoaked seeds, MSP with clay and MSP with diatomaceous clay) were sown in pots under upland conditions by placing 20 dry or presoaked seeds, or 5 MSP with either clay or diatomaceous clay on the soil surface of each pot in the presence of the legume living mulch (dry weight of legume stems and leaves = 217 g m⁻²). After sowing, the pots were dipped in a plastic box (area, 36.1 × 51.3 cm; height, 30.1 cm) filled with water in order to flood the soil. The water level was maintained at 10 cm above the soil surface to suppress legume growth and moisten the seeds; it was drained after 3 days. The pots were arranged outside the greenhouse in a randomized complete block design, and each treatment was replicated 4 times. The pots were irrigated regularly, as required.

Rice seedlings were harvested at 16 DAS and the number of seedlings per pot, shoot length of seedling, leaf chlorophyll content, plant age in leaf number, and leaf and root dry weight were measured. The leaf chlorophyll content was measured with a chlorophyll meter (SPAD 502, Minolta Co. Ltd. Osaka, Japan). The rice seedlings that developed beyond the two-leaf stage (incomplete leaf was defined as the first-leaf) were defined as established. The seeds that did not germinate or elongated at 16 DAS were collected and incubated at 30°C in petri dishes containing distilled water (10 ml) in order to evaluate seed survival.

5. Statistical analysis

All data were analyzed by Scheffe’s F-test (p=0.05) with Statcel software (OMS publishing Inc., Saitama, Japan).

Results and Discussion

1. Germination of seeds incubated in a petri dish

Fig. 1 shows the germination rates of the 4 seed types in petri dishes under laboratory conditions. After 3 days of incubation, the rate of seed germination was 11% in the dry seed, 99% in the presoaked seed, 54% in the MSP with clay, and 37% in the MSP with diatomaceous clay. The rate significantly (p=0.05) differed with the seed type. After 5 days, the rate of germination was highest in the presoaked seed and was significantly (p=0.05) higher than that of the MSP with clay and MSP with diatomaceous clay. The rates in the dry seed, MSP with clay, and MSP with diatomaceous clay were 86%, 78%, and 75%, respectively; there was no significant difference among these rice seed types.

The presoaked seeds emerged earlier than the dry seeds and MSPs. Although the MSP was comprised of presoaked seeds, seedling emergence occurred later than in the presoaked seed; this was probably due to the time required for water to enter through the clay coating.

2. Establishment and growth of seedlings sown on the potted soil covered with living mulch

After flooding for 3 days to suppress legume growth, the legume leaf color turned from green to brown within 5 days and then the plants withered slowly under the well-drained conditions in the pot experiment. The MSP with diatomaceous clay exhibited the highest seedling establishment rate
(55%) among the rice seed types at 16 DAS in the pot experiment, followed by the MSP with clay (50%), the dry (40%), and the presoaked seed (5%) as shown in Fig. 2. The establishment rate in the presoaked seed was significantly ($p=0.05$) lower than that in all the others, and there was no significant difference between dry seeds, MSP with clay, and MSP with diatomaceous clay (Fig. 2).

The seeds that had not germinated or elongated at 16 DAS in the pot experiment were collected and evaluated for seed survival rate by the incubation test; however, no seeds survived. Consequently, the rates of plant death by 16 DAS in the dry seeds, presoaked seeds, MSP with clay, and MSP with diatomaceous clay were 60%, 95%, 50%, and 45% of the sown seeds, respectively (Table 1).

The shoot length of seedlings, plant age in leaf number, top and root dry weight of seedlings, and the top-root ratio did not differ significantly ($p=0.05$) among the seed types, while the leaf chlorophyll
contents of the seedlings from dry seeds and MSP with diatomaceous clay were significantly ($p=0.05$) higher than those of the seedlings from presoaked seeds in the pot experiment. Shoot length and top and root dry weight of seedling were greatest in the seedlings from dry seed and lowest in those from the presoaked seeds; however, the differences were non-significant. Leaf chlorophyll content and plant age in leaf number were highest in the seedlings from MSP with diatomaceous clay and lowest in those from the presoaked seeds. Moreover, the top-root ratio was highest in the seedlings from presoaked seed and lowest in those from the MSP with diatomaceous clay. Therefore, at 16 DAS, the seedlings from the dry seeds and MSP with diatomaceous clay were more vigorous than those from the presoaked seed.

The rates of seedling establishment in the MSP with either clay or diatomaceous clay were significantly ($p=0.05$) higher (45% and 50%, respectively) than in the presoaked seed, and 10% and 15% higher, respectively, than in the dry seed. Thus, seed coating with clay, improved seedling establishment, although no significant differences were observed among the dry seed, MSP with clay, and MSP with diatomaceous clay in the pot experiment.

Nakano and Sugimoto (1999) suggested that the low light intensity under the canopy of some green manures led to the spindly growth (low leaf chlorophyll content and high top/root ratio) of the rice seedlings. This resulted in a low rate of rice seedling establishment. In their studies, flooding was carried out 1 or 2 months after sowing to initiate mulch decomposition. In order to avoid the negative impacts on the growth of rice seedling reported in the previous study, we conducted flooding for 3 days from 1 to 3 DAS. The flooding suppressed legume growth, and consequently, the spindly growth of rice seedlings was not observed in the present pot experiment.

In some studies, organic matter application promoted the release of organic acids from straw and green manure (Motomura, 1961, 1962; Gotoh and Onikura, 1971) and from organic waste in flooded soils (Ueno and Suzuki, 2005). Moreover, organic acids inhibited the root growth of rice (Takijima et al., 1960). Mineta et al. (1996) measured pH and EC of the flooded water in pots covered with living white clover mulch and conducted a lettuce seed bioassay, to evaluate the allelopathic potential of aqueous leachate from white clover mulch. They reported that in the flooded water in pots covered with white clover, pH decreased and EC increased from 1 day after flooding, compared with those in the absence of white clover. Furthermore, the flooded water inhibited hypocotyl growth of lettuce seedlings from 2 days after flooding. These results strongly suggested release of growth inhibitors such as organic acids from the white clover mulch. Therefore, in our pot experiment, the reduced rate of seedling establishment was possibly attributed to the inhibitors produced during the decomposition of the legume under the flood-induced anaerobic conditions from 1 to 3 DAS. Meanwhile, under aerobic conditions during the drainage (from 4 to 16 DAS), the quantity of inhibitors such as organic acids generated from the legume decomposition may decrease.

The rate of seed germination under laboratory conditions was 99% in the presoaked seed, and 11%-54% in the other types of seeds after 3 days of incubation (Fig. 1). Thus, most of the presoaked seeds germinated during flooding in the pots with living mulch. The rates of establishment and growth of seedlings from the presoaked seed were extremely low (Fig. 2, Table 1). The reason for this low rate is not clear, but it may be related to the seedling exposure to harmful substances during flooding and the generation of rice growth inhibitors from the decomposed green manure in the pot experiment. The timing of flooding and sowing seems to seriously affect seedling establishment and growth in rice directly seeded on paddy soil covered with legume mulch. The dry seeds and MSPs are more suited to direct seeding on paddy fields covered with legume mulch than the presoaked seeds if the field is flooded for 3 days after seeding.

In direct sowing on well-drained paddy fields, weed control is one of the most difficult problems (Tomihisa, 1993). The combination of this cultivation system and a cover crop may be effective for the control of weed growth because cover crops suppress weed growth (Miura and Watanabe, 2002). However, certain adverse consequences, i.e., low light intensity under the canopy of the cover crop and the generation of rice growth inhibitors from cover crop decomposition under flood-induced anaerobic conditions, affect rice growth negatively. The seed coating with clay described here appears to be effective in overcoming the disadvantages associated with cover crop use; however, further studies in both pot and field are necessary for the development of materials to coat seeds, and to evaluate cultivation management (duration of flooding, drainage, and sowing), lodging resistance, yield, and the quality of rice.

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