Improvement of Rice Seedling Establishment on Sulfate-Applied Submerged Soil by Seed Coating with Poorly Soluble Molybdenum Compounds

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Abstract: Although direct seeding is expected to be a labor-saving method in rice cultivation, poor seedling establishment is a major obstacle to widespread use of this practice. I previously reported that the seedling establishment of rice sown on sulfate-applied submerged soil was improved by the application of potassium molybdate to the soil. In this study, I investigated the effects of seed coating with poorly soluble molybdenum compounds on the seedling establishment to reduce the necessary amount of molybdenum (Mo). Seed coating with poorly soluble Mo compounds improved the seedling establishment in sulfate-applied submerged soil. Especially, ammonium phosphomolybdate and potassium phosphomolybdate were more effective and difficult to impair the seedling establishment even by seed coating of excessive amounts. Mo trioxide, which is a cheapest Mo compound, was also effective. Accordingly, seed coating with these Mo compounds would have the potential to improve rice seedling establishment in sulfur-rich submerged soil.

Key words: Direct seeding, Molybdenum, Paddy soil, Rice plants, Seed coating, Seedling establishment, Sulfide.

Poor establishment is a major obstacle to the practice of direct seeding of rice plants (Oryza sativa L.) (Yamauchi, 1997; Furuhata, 2009). The impairment of the germination and subsequent growth of rice seeds occurring after application of ammonium sulfate, a nitrogen (N) fertilizer, has been suggested to be caused by the sulfide ions generated from sulfate ions when soil is reduced (Osugi and Kawaguchi, 1938; Kawaguchi, 1944; Hara, 2013a). Previously, I reported that the application of molybdate could improve rice seedling establishment in sulfate-applied submerged soil by suppressing the generation of harmful sulfide ions derived from sulfate ions (Hara, 2013b). The application of molybdate might improve seedling establishment, especially in sulfur-rich paddy fields. However, a large amount of molybdate may be required when applied uniformly to the soil of paddy fields.

In this study, I examined how seedling establishment would be affected by the effect of coating of seeds with molybdenum (Mo) compounds which was expected to require less Mo than the application to the soil. Only poorly soluble Mo compounds were used because the suppression of rice growth was observed when soluble potassium molybdate was applied to the soil in large amounts (Hara, 2013b). The aim of this study was to find the most appropriate kind and amount of Mo compounds for the seed coating. First, I examined the effect of seed coating using various kinds of Mo compounds on seedling establishments under an accessible condition (at 30°C, using swollen seeds, under a fluorescent lamp). Next, I examined the effects of selected Mo compounds under different temperatures (at 20 or 30°C) and different degrees of seed soaking.

Materials and Methods

Swollen rice seeds (cv. Hinohikari) were prepared by sterilizing in 70% ethanol and 5-fold diluted solution of commercial sodium hypochlorite solution (available chlorine > 5%) for 10 min each, and then soaked in 10°C water for 5 d and then in 30°C water for about 2 d. Any germinated seeds were removed and only ungerminated seeds were used (referred to as “swollen seeds” hereafter). These seeds were soaked in a polyvinyl alcohol solution containing a 10-fold diluted commercial laundry detergent for 1 min, spin-dried, and then coated with 7 poorly soluble Mo compounds of 0 – 2 mol Mo kg⁻¹ air-dried...
seeds (expressed as mol Mo kg\(^{-1}\) hereafter). In a preliminary experiment, soaking in polyvinyl alcohol solution did not affect the seedling growth. Mo compounds used for coating were Mo metal powder (referred to as MoM hereafter), Mo trioxide (MoO), molybdic acid (MoH), magnesium molybdate (MoMg), calcium molybdate (MoCa), ammonium phosphomolybdate (MoPNH), and potassium phosphomolybdate (MoPK).

Moist soil (fine-textured gray lowland soil, light clay) was obtained from the topsoil (ca. 0 – 10 cm in depth) of a paddy field in Chikugo, Fukuoka Prefecture, Japan. The water content of the soil was about 25% by weight. The available sulfur content of the soil used was 2.0 mmol kg\(^{-1}\) (Hara, 2013a). The soil was passed through a 10-mm sieve without previous drying and then refrigerated until use to minimize changes in soil conditions (Hara, 2013b). Wet soil equivalent to 100 g of dry soil was placed in pots (8 cm in diameter, 8 cm high). A solution containing ammonium sulfate (corresponding to 4 mmol kg\(^{-1}\) dried soil), and potassium chloride (corresponding to 1 mmol kg\(^{-1}\) dried soil) was added to each pot until the total amount of water was 1.5 times the weight of dry soil. The applied amount of ammonium sulfate corresponded to 11 g N m\(^{-2}\), assuming that 100 kg dried soil corresponded to 1 m\(^2\), although the generally applied amount of N is around 5 g N m\(^{-2}\). This was to make the effect of Mo compounds clear by promoting the generation of sulfide ions from applied sulfate ions. The mixture was shaken for 1 hr and then kept in a refrigerator for 2 d.

Eight swollen seeds coated with each Mo compound were sown at a soil depth of 15 mm in each pot (Hara, 2013b). The pots were placed in growth chambers at 30ºC with fluorescent lamp (ca. 0.1 mmol m\(^{-2}\) s\(^{-1}\), 12 hr d\(^{-1}\)) to check whether the result is similar to that under low intensity fluorescent light. Six pots were used for each treatment.

The water level was maintained at about 15 mm. The rate of seedling establishment was measured 3 – 4 wk after sowing. Seedling establishment was determined by the appearance of the third leaf. The rate of emergence on the soil surface was not measured because it was hardly affected by the application of sulfate or molybdate in a previous study (Hara, 2013b). The solubility and pH value of each Mo compound used for seed coating were examined as follows (Table 1). Each Mo compound was suspended in distilled water at a ratio of 0.1 mol Mo L\(^{-1}\) at room temperature (ca. 25ºC), and then centrifuged at 10,000 rpm for 10 min. These supernatant solutions were 10 – 100-fold diluted and the Mo concentrations in these diluted solutions were analyzed using inductively coupled plasma atomic-emission spectroscopy (ICP-AES; Varian Vista AX). In addition, pH values of the water suspension of these Mo compounds were also examined by the same method. This was done to imitate pH neutralization in paddy soil.

### Results

Swollen seeds were coated with each Mo compound, sown on submerged soil, and incubated at 30ºC. As a result, the rate of seedling establishment was improved (more than 40%) when seeds were coated with MoO, MoH, MoPNH, or MoPK at 0.05 – 2 mol Mo kg\(^{-1}\), MoMg at 0.05 – 1 mol Mo kg\(^{-1}\), MoCa at 1 mol Mo kg\(^{-1}\), or MoM at 0.05 or 2 mol Mo kg\(^{-1}\), although the rates were less than 20% when seeds were not coated (Fig. 1a). The

### Table 1. Solubilities and pH values of Mo compounds without/with magnesium oxide.

| Mo compounds\(^{(a)}\) | Without magnesium oxide\(^{(b)}\) | With magnesium oxide\(^{(c)}\) |
|------------------------|-------------------------------|-----------------------------|
|                        | Mo (mM)\(^{(d)}\) | pH\(^{(e)}\) | Mo (mM)\(^{(d)}\) | pH\(^{(e)}\) |
| MoM                   | 2.9             | 3.5            | 9.3            | 9.8            |
| MoO                   | 8.1             | 2.5            | 101            | 5.9            |
| MoH                   | 8.8             | 3.6            | 102            | 8.8            |
| MoMg                  | 92              | 6.1            | 94             | 9.7            |
| MoCa                  | 1.2             | 7.4            | 1.1            | 10.2           |
| MoPNH                 | 0.2             | 4.0            | 97             | 6.1            |
| MoPK                  | 4.2             | 3.2            | 99             | 6.2            |

\(^{(a)}\) MoM: Mo metal powder, MoO: Mo trioxide, MoH: molybdic acid, MoMg: magnesium molybdate, MoCa: calcium molybdate, MoPNH: ammonium phosphomolybdate, MoPK: potassium phosphomolybdate. \(^{(b)}\) Each Mo compound at 0.1 mol Mo was mixed per distilled water of 1 L without the addition of magnesium oxide. \(^{(c)}\) Each Mo compound of 0.1 mol Mo was suspended in 1 L of distilled water together with equimolar magnesium oxide to raise the pH. \(^{(d)}\) Mo concentration in the supernatant of the suspension. \(^{(e)}\) pH value of the suspension.
improvement of seedling establishment by MoCa and MoM was smaller than that by the other compounds. Coating with MoO, MoH, or MoMg at a higher rate (0.5 – 2 mol Mo kg\(^{-1}\)) had a weaker improvement effect. However, the effect of coating with MoPNH or MoPK was not weakened even at 2 mol Mo kg\(^{-1}\).

In the following experiments, swollen seeds coated with MoO, MoPNH or MoPK were incubated under a fluorescent lamp at 20ºC (Fig. 1b), and unswollen seeds were incubated at 30ºC (Fig. 1c) or 20ºC (Fig. 1d). In addition, swollen seeds coated with these compounds were incubated at 20ºC under a metal halide lamp (0.4 mmol m\(^{-2}\) s\(^{-1}\)) instead of a fluorescent lamp (0.1 mmol m\(^{-2}\) s\(^{-1}\)) (Fig. 1e). The rates of seedling establishment were significantly increased by coating with MoO, MoPNH, or MoPK at about 0.05 – 2 mol Mo kg\(^{-1}\) under these conditions. However, the improvement of the seedling establishment was sometimes weakened when the amount of Mo exceeded 0.2 – 2 mol Mo kg\(^{-1}\).

**Discussion**

1. **Effect of different Mo compounds on seedling establishment**

   The seedling establishment of rice plants in sulfate-applied submerged soil was improved by coating the seed with most of the procurable poorly soluble Mo compounds when swollen seeds were used and incubated at 30ºC with weak lights (Fig. 1a). These poorly soluble Mo compounds on seeds may release molybdate ions slightly when coated seeds are sown in submerged soil. Molybdate ions may inhibit the generation of harmful sulfide ions from sulfate ions and improve the seedling establishment, as well as when soluble potassium molybdate was applied to the submerged soil (Hara, 2013b). Even MoM, which is not a molybdate salt, had an improvement effect, implying that the surface of Mo metal may be oxidized and that molybdate ions may be slightly released from Mo oxide on the surface of Mo metal. This was supported by the results that the pH of the MoM-suspended solution was acidic and the molybdate concentration increased slightly by the
addition of magnesium oxide (Table 1). MoO, MoH and MoMg had a higher solubility than the other compounds (Table 1). The improvement effect on seedling establishment was higher when Mo was used at 0.2 – 0.5 mol Mo kg\(^{-1}\) (Fig. 1a). Then, it weakened with the increase in the amount of Mo. In particular, the most soluble MoMg showed the largest decrease in seedling establishment when the amount of Mo was high. This implies that excess Mo may weaken the improvement effect. On the other hand, the improvement effect of MoPNH and MoPK was maintained even when the amount of Mo was high. This may be because the solubility of MoPNH and MoPK was less than other compounds.

The improvement effects of MoM or MoCa were remarkably lower than those of the other Mo compounds (Fig. 1a). This may be because of their low solubility (Table 1). The low solubility of MoM may be because molybdate ions were derived only from the small amount of Mo oxide on the metal surface. The low solubility of MoCa may be caused by its chemical property. The improvement effects of MoPNH or MoPK were high although their solubility was low. This may be because their solubility increased with the increase in pH (Table 1). The pH around seeds coated with MoPNH or MoPK may increase to pH 6 – 7 after the seeds were sown in soil because soil has a high buffering effect of pH. Phosphomolybdate salts such as MoPNH and MoPK may degrade into more-soluble molybdate salt at pH 5 – 6 (Tsigdinos, 1974). Therefore, MoPNH and MoPK could supply a modest amount of molybdate ions in soil although their solubility was low. Furthermore, even excess coating with MoPNH or MoPK may not impair the improvement effect because their solubilities are originally low. Nonetheless, all Mo compounds, except for MoM and MoCa, improved seedling establishment when seeds were coated with them at an appropriate amount, 0.05 – 0.5 mol Mo kg\(^{-1}\).

2. Availability of seed coating with Mo compounds in rice cultivation

The seedling establishment was improved by seed coating with MoO, MoPNH, or MoPK at a temperature of 20 or 30°C and light strength of 0.1 or 0.4 mmol m\(^{-2}\) s\(^{-1}\) during the incubation (Fig. 1a – e). It is considered that the effect of each Mo compound on seedling establishment would be stable regardless of temperature, seed swelling before coating, or light intensity. Accordingly, these Mo compounds are expected to be applicable to improve seedling establishment in the direct seeding of rice in paddy fields where sulfide ions are prone to generate.

In this study, the amount of ammonium sulfate applied to soil (ca. 10 g N m\(^{-2}\)) was larger than the commonly applied amount. However, the seedling establishment was impaired by ammonium sulfate applied at a rate of more than 2 – 4 g N m\(^{-2}\) (Yamamoto et al., 2004; Hara, 2013a), which was comparable to the general amount of N application. Accordingly, it is expected that the seed coating with these Mo compounds would be effective when ammonium sulfate was applied as the basal fertilizer. When sulfate was not applied, sulfide ions seemed to generate from the sulfate ions originally contained in the soil. The available sulfur content of the soil used was 2.0 mmol kg\(^{-1}\) (Hara, 2013a). However, it is difficult to tell whether this sulfur level was high or not, due to the limited availability of information at this time. Anyway, the seed coating with these Mo compounds might be effective in sulfur-rich paddy fields even when ammonium sulfate was not applied.

The amount of Mo necessary for seed coating to improve the seedling establishment may be roughly 0.1 mol Mo kg\(^{-1}\) seeds (Fig. 1). If seeds of 3 kg were sown in a paddy field of 10 a (1000 m\(^{2}\)), the necessary amount of Mo was 0.3 mol Mo per 10 a. In contrast, when the Mo compound was applied to paddy soil before seed sowing to improve the seedling establishment similarly, the necessary amount of Mo may be roughly 2 mmol kg\(^{-1}\) dried soil (Hara, 2013b). This means that 200 mol Mo per 10 a of paddy field was necessary, assuming that dried soil of 100 kg existed in paddy field of 1 m\(^{2}\). Accordingly, the necessary amount of Mo was remarkably less for seed coating rather than application to soil. The necessary amount of MoO, which may be the cheapest Mo compounds, for seed coating of 10 a was 43 g (0.3 mol Mo), and the cost was 130 yen, assumed that MoO of 1 kg was 3,000 yen. This cost is so cheap that the seed coating with Mo compound could be economical for actual rice cultivation.

In addition, Mo is an essential micronutrient for plants and is sometimes used as a fertilizer (Marschner, 1995; MacLeod et al., 1997). According to the general fertilization method, sodium molybdate or ammonium molybdate of 30 – 50 g (about 0.1 – 0.3 mol Mo) per 10 a should be applied to Mo-deficient crops (Takahashi et al., 1980). The used amount of Mo compound for seed coating is not more than that for general fertilization method. Accordingly, the usage of Mo compound for seed coating may be little of the adverse impacts.

In the future, it is necessary to investigate the effects of seed coating with Mo compound on seedling establishment, growth and harvest under different conditions. Especially, the relationship between the effect of the seed coating with these Mo compounds and the sulfur content of the paddy field should be investigated for the appropriate use of Mo compounds.

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