Improvement of Soybean Seedling Establishment under a Flooded Condition by Seed Coating with Molybdenum Compounds

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Abstract: In Japan, soybeans are generally cultivated in paddy fields which often have poor drainage. West of the Kanto region, the seeds are sown in the rainy season. Therefore, the seedling establishment is prone to damage by the rain after sowing. The generation of sulfide ions in flooded and reduced soil is thought to be one factor causing this flooding damage. Since molybdate ions suppress the generation of sulfide ions, the effect of coating soybean seeds with molybdenum compounds, on the seedling establishment under a flooded condition was examined. Soybean seeds were coated with several kinds of poorly-soluble molybdenum compounds at different concentrations. The coated seeds were sown in soil and then flooded for 3 d at 25 – 30ºC. Seedlings were not established from seeds not coated with molybdenum compounds. Seedling establishment was appreciably improved by coating the seeds with a molybdenum compound at the rate of 0.5 – 1 mol-Mo kg\(^{-1}\). Seedling establishment was especially improved by coating with molybdenum trioxide. Accordingly, coating the soybean seed with molybdenum compounds could mitigate the flooding damage occurring after sowing.

Key words: Flooding damage, Molybdenum compound, Seed coating, Seedling emergence, Seedling establishment, Soybean, Submergence.

In Japan, soybeans \([Glycine max (L.) Merr.]\) are generally cultivated in paddy fields which often have poor drainage. West of the Kanto region, they are sown in the rainy season. The heavy rain after sowing causes flooding of the soybean field and the soybean seeds rot. Such flooding damage hinders seedling establishment and lowers the yield. Much research has been conducted on the flooding damage of soybean seedlings. Zheng and Watanabe (2000) reported that the sugar exudation from imbibing soybean seeds caused the failure of seedling establishment. Nakayama et al. (2005) reported that a rapid inrush of water into soybean seeds caused the physical disruption leading to a marked reduction in seedling establishment. Kokuryu et al. (2010) reported that the inhibition of the seedling establishment was attributed to the imbibition damage, soil microorganisms and soil surface strength. Kato et al. (2013) reported that microorganisms especially oomycetes are involved in the flooding damage. Accordingly, these problems need to be solved to reduce the flooding damage of soybean seedlings.

Rice plants can establish seedlings on flooded soil. The soil around sown seeds is rapidly reduced to the level causing generation of harmful sulfide ions, even if it was 20ºC as in spring (Hara, 2013a). The establishment of rice plants is impaired by the generation of the sulfide ions. Such rapid generation of sulfide ions at a cool temperature around rice seeds implies that generation of sulfide ions might impair the seedling establishment of also soybean seeds flooded. The generation of sulfide ions in flooded and reduced soil may be one factor causing the flooding damage. The application and seed coating of molybdenum compounds improve the establishment of rice seedling because the molybdate ions suppress the generation of sulfide ions (Hara, 2013b; 2013c). Thus, flooding damage of soybeans might be mitigated by the suppression of sulfide ion generation using molybdenum compounds.

In this study, the effect of seed coating with molybdenum compounds, which release molybdate ions, on the flooding damage of soybean seedling establishment was investigated.

Materials and Methods

1. Effect of seed coating with different amounts of molybdenum compounds on seedling establishment under a flooded condition (Exp. 1)

Air-dried soybean seeds (cv. Fukuyutaka) were coated with one of three poorly-soluble molybdenum compounds; i.e., molybdenum trioxide (MoO), ammonium phosphomolybdate (MoPNH) and potassium phosphomolybdate (MoPK). Each molybdenum compound at 0, 0.02, 0.5, 0.1, 0.2, 0.5, 1, or 2 mol-Mo kg\(^{-1}\) (per air-dried seeds) was mixed with polyvinyl
alcohol powder of 2% weight of the molybdenum compound. Soybean seeds were coated with the mixed powder using a water mist.

Moist soil (fine-textured gray lowland soil, light clay) was obtained from the toposoil (ca. 0 – 10 cm in depth) of a paddy field located in Chikugo, Fukuoka Prefecture, Japan. The water content of the soil was about 25% by weight. The soil was passed through a 10-mm sieve without previous drying and then refrigerated until use to minimize changes in soil conditions. Wet soil equivalent to 100 g of dry soil was placed in pots (8-cm in diameter, 8-cm high, glass, without holes at the bottom). 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 0.7 times the weight of dry soil. The applied amount of ammonium sulfate corresponded to 11 g m\(^{-2}\) nitrogen, assuming that 100 kg dried soil corresponds to 1 m\(^{2}\). The soil surface was made flat by pressing with a board.

Eight seeds coated with each amount of each molybdenum compound were sown on the soil surface and were pressed down using a board until the top of seeds was at the soil surface. The pots were placed in a growth chamber, in which the air temperature was controlled at 25°C, and illuminated with a metal halide lamp (ca. 400 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), 12 h d\(^{-1}\)). Then, water was added to the pot and water level was maintained at about 15 mm above the soil surface. Then, seeds and soil surface were pressed down slightly several times using a board until the seeds were covered by the soil. Three days after sowing, water on the soil surface was removed using a pipette. Then the soil gradually became dry. Only enough water to be absorbed by soil was added when the bottom soil became dry. Rates of seedling establishment were measured about 3 wk after sowing. Seedling establishment was determined as mentioned above. Six pots were examined for each condition. The temperature around the pot was about 25°C at night and about 30°C in the daytime with illumination from a metal halide lamp.

2. Effect of seed coating with various molybdenum compounds on seedling establishment under a flooded condition (Exp. 2)

As mentioned above, soybean seeds were coated with one of four poorly-soluble molybdenum compounds; i.e., MoO, molybdic acid (MoH), MoPNH and MoPK, at 0.5 mol-Mo kg\(^{-1}\). These coated seeds and uncoated seeds were sown in the pots and were flooded in the growth chamber. Two days after sowing, water on the soil surface was removed. Seedling establishment was determined as mentioned above. Six pots were used for each condition. Two experiments were carried out at different times. According to two-way analysis of variance, the establishment rate of seedling showed a significant difference only with seed coating (\(p = 0.00\)) but not between the two experiments (\(p = 0.20\)) or the interaction (\(p = 0.66\)). Accordingly, the average values were calculated from the results of two experiments.

3. Effect of seed coating with a molybdenum compound on seedling establishment after different flooding periods (Exp. 3)

Soybean seeds were coated with MoPK at 0.5 mol-Mo kg\(^{-1}\) seeds. Pots containing soil were prepared as in Exp. 1, except that ammonium sulfate was not applied to the soil. Similarly, the coated seeds and uncoated seeds were sown in the pots and were flooded in the growth chamber. Water on the soil surface was removed 0 d (after seeds were sown) 1, 2 or 3 d after sowing. Seedling establishment was determined as mentioned above. Six pots were used for each condition.

4. Effect of seed coating with a molybdenum compound on seedling establishment under a non-flooded condition (Exp. 4)

Soybean seeds were coated with MoPK at 0.00, 0.02, 0.05, 0.1, 0.2, 0.5, 1, or 2 mol-Mo kg\(^{-1}\). Pots containing soil were prepared as in Exp. 1, and the coated seeds or uncoated seeds were sown in the pots. However, soil was not flooded. Only enough water to be absorbed by soil was added when the bottom of soil was dried. The number of the seedlings, which the unifoliolate leaf appeared, was measured every day in the growth chamber. The daily change in the number of the seedlings which showed appearance of the unifoliolate leaf, was fitted to the Richards function (Hara, 1999) and the mean period until appearance of unifoliolate leaf was calculated. Six pots were examined for each condition.

Results

1. Effect of seed coating with different amounts of molybdenum compounds on seedling establishment under a flooded condition (Exp. 1)

Uncoated seeds flooded for 3 d, decayed and seedling establishment was not observed (Fig. 1). However, the seeds coated with a molybdenum compound, MoO, MoPNH or MoPK, at 0.2 – 2 mol-Mo kg\(^{-1}\) seed, germinated and the seedlings were established after flooding for 3 d as shown in Fig. 1. The optimum coating rate was 0.5 mol-Mo kg\(^{-1}\) for all molybdenum compounds examined, and the establishment rate increased to about 70, 50 and 70% by coating with MoO, MoPNH and MoPK, respectively.

2. Effect of seed coating with various molybdenum compounds on seedling establishment under a flooded condition (Exp. 2)

Uncoated seeds flooded for 2 d decayed and seedling
Seeds were coated with MoO, MoPNH, or MoPK. Sown seeds were flooded for 3 d. Error bars represent standard errors (n = 6). Different alphabets mean significant difference at 5% (Tukey’s multiple comparison).

Fig. 1. Effects of seed coating with different amounts of molybdenum compounds on seedling establishment rates under a flooded condition.

Seeds were uncoated (No), or coated with MoO, MoH, MoPNH, or MoPK at 0.5 mol-Mo kg$^{-1}$. Sown seeds were flooded for 2 d. Error bars represent standard errors (n = 12). Different alphabets mean significant difference at 5% (Tukey’s multiple comparison).

Fig. 2. Effects of seed coating with different kinds of molybdenum compounds on seedling establishment rates under a flooded condition.

Seeds were uncoated (No), or coated with MoO, MoH, MoPNH, or MoPK at 0.5 mol-Mo kg$^{-1}$. Sown seeds were flooded for 2 d. Error bars represent standard errors (n = 6). *p < 0.05 vs. samples lacking molybdenum compounds (Dunnett’s multiple comparison).

Establishment was not observed at all (Fig. 2). However, the establishment rate was significantly increased by coating the seed with MoO, MoH, MoPNH or MoPK (Fig. 2). The establishment rate of seeds coated with MoO was about 70% and was significantly higher than that of seeds coated with any other molybdenum compound examined.

Fig. 3. Effects of seed coating with a molybdenum compound on seedling establishment rates after different flooding periods.

Seeds were uncoated (No) or coated with MoPK at 0.5 mol-Mo kg$^{-1}$. Error bars represent standard errors (n = 6). **p < 0.01 vs. uncoated samples (t-test).

3. Effect of seed coating with a molybdenum compound on seedling establishment after different flooding periods (Exp. 3)

The seedling establishment of uncoated seeds with temporarily flooding (flood period, 0d) was about 70% (Fig. 3). However, after flooding for 1 – 3 d, the establishment rate was less than 5%. In soybean seeds
coated with MoPK (0.5 mol-Mo kg⁻¹), the seedling establishment rate with temporarily flooding was about 60%, which was not significantly different from that in uncoated seeds with temporarily flooding. In the seeds coated with MoPK, the establishment rate was more than 20% even after 1–3 d flooding. The establishment rate was significantly higher than in uncoated seeds.

4. Effect of seed coating with a molybdenum compound on seedling establishment under a non-flooded condition (Exp. 4)

In uncoated soybean seeds, the seedling establishment rate without flooding was 100% (Fig. 4). In the seeds coated with MoPK at a concentration of up to 0.5 mol-Mo kg⁻¹, the seedling establishment rate was also about 100%. In the seeds coated with MoPK at 1 and 2 mol-Mo kg⁻¹, the seedling establishment rate was about 90 and 50%, respectively.

Fig. 5 shows the days from sowing to appearance of unifoliolate leaves in the seeds coated with MoPK at different rates. The days to appearance of unifoliolate leaf did not increase significantly when the amount of MoPK was up to 0.2 mol-Mo kg⁻¹. However, it was increased significantly to about 8 d when the amount of molybdenum compound was 0.5 or 1 mol-Mo kg⁻¹, and it was about 10 d when the amount was 2 mol-Mo kg⁻¹.

Discussion

The establishment rate sometimes differed with the experiment for the same treatment (ex. Fig. 1 vs. Fig. 3). This suggested that the experimental conditions were not strictly the same in these experiments. One reason for the difference might have been sowing depth. The soybean seeds were sown on the soil and the soil surface was pressed using a board until seeds were covered with soil. Seedling depth on soybean seeds was so shallow that even a slight difference in seedling depth might have influenced the seedling establishment. Seedling depth might have differed with the experiment.

In the soybean seeds not coated with molybdenum compounds, seedling establishment was not observed at all (Fig. 1). However, in the seeds coated with a molybdenum compound at the rate of 0.2–2 mol-Mo kg⁻¹, the seedling establishment was improved. At a coating rate of 0.5–1 mol-Mo kg⁻¹, the seedling establishment was remarkably improved regardless of the kind of molybdenum compounds. These results indicate that the seed coating with molybdenum compounds improved the impaired establishment of seedlings flooded for a few days, as well as in the rice seeds sown in flooded soil (Hara, 2013c).

Molybdate ions inhibit the generation of sulfide ions (Hori et al., 1990; Biswas et al., 2009; Hara, 2010b; 2013c). The result that the coating with molybdenum compounds improved the establishment of flooded soybean seeds implies that the generation of sulfide ions may be an important factor inhibiting the establishment of flooded soybean seedling, as in rice seedlings. In this study, the soil temperature rose to about 30°C in the daytime with illumination by metal halide lamps, thus promoting the soil around seeds to be reduced enough for sulfide ions to be generated. The soybean seeds are larger than rice seeds and elute sugars (Zheng and Watanabe, 2000), which may

Fig. 4. Effects of seed coating with different amounts of a molybdenum compound on seedling establishment rates under a non-flooded condition.

Seeds were coated with MoPK. Error bars represent standard errors (n = 6). **p < 0.01 vs. samples lacking molybdenum compounds (Dunnett’s multiple comparison).

Fig. 5. Effects of seed coating with different amounts of a molybdenum compound on the mean period from sowing to appearance of unifoliolate leaf under a non-flooded condition.

Seeds were coated with MoPK. Error bars represent standard errors (n = 6). **p < 0.01 vs. samples lacking molybdenum compounds (Dunnett’s multiple comparison).
promote the multiplication of microorganisms and the reduction of soil around the seeds.

Sulfate ions, which may change to sulfide ions, were not added in Exp. 3. Nonetheless, the seedling establishment was improved much by the seed coating with the molybdenum compound (Fig. 3). It may be because sulfide ions generated from the sulfate ions that existed in soil originally. However, the result did not deny the possibility that the improvement might also be caused by a mechanism other than the inhibition of the generation of sulfide ions. For example, the molybdenum compound might improve the seedling establishment by directly inhibiting the multiplication of microorganisms like the microbicide, because Kato et al. (2013) reported that a microbicide improved the establishment of soybean seedlings under a flooded condition.

Nakayama et al. (2005) pointed out a difference in the flooding damage of soybean before and after radicle protrusion. The flooding damage of soybean before radicle protrusion seems to be mostly caused by the physical disruption through rapid water absorbance, the decomposition due to microorganism and so on (Nakayama et al., 2005; Kokuryu et al., 2010; Kato et al., 2013). The flooding damage of soybean after radicle protrusion seems to be mostly caused by oxygen deficiency, hardness of soil surface and so on (Nakayama et al., 2005; Kokuryu et al., 2010). In this study, soybean seeds were flooded soon after sowing, which indicates that coating of seed with a molybdenum compound would be effective against the flooding damage before radicle protrusion. In addition, the soybean seeds were sown just below the soil surface in this study. The coating of seeds with molybdenum compound might not improve the impairment of establishment caused by hardness of soil surface because the sowing depth was shallow in this experiment. Accordingly, additional validations would be necessary for assessing whether the coating with a molybdenum compound is effective against flooding damage in actual soybean cultivation.

The effect of the coating with a molybdenum compound on the soybean establishment after flooding decreased with the increase in the molybdenum compound above 0.5 mol-Mo kg$^{-1}$ regardless of the kind of molybdenum compound (Fig. 1). The seedling establishment under a non-flooded condition was also inhibited by coating with a molybdenum compound above 0.5 mol-Mo kg$^{-1}$ (Fig. 4). In addition, the growth rate under a non-flooded condition was also delayed by coating the seeds with a molybdenum compound at a rate of 0.5 mol-Mo kg$^{-1}$ or higher (Fig. 5). Accordingly, coating the seed with a molybdenum compound would improve the seedling establishment under a flooded condition but inhibit the growth of soybean at a high concentration. The appropriate amount of molybdenum compound was about 0.5 mol-Mo kg$^{-1}$ (Fig. 1).

All 4 molybdenum compounds examined improved seedling establishment under a flooded condition (Fig. 2). MoO was the most effective, but the reason was uncertain. At the current cost of about 3,000 yen kg$^{-1}$, which is the lowest of the molybdenum compounds examined, the cost for the coating would be about 200 yen kg$^{-1}$ at the coating rate of 0.5 mol-Mo kg$^{-1}$. Thus coating the seeds with MoO may be a practical method for soybean cultivation.

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