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Ecophysiology of Seed Germination in Chinese Milk Vetch (*Astragalus sinicus* L.)

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ABSTRACT : Germination and emergence habits of Chinese milk vetch (CMV) were examined to obtain the basic information for the effect of environmental conditions and cultural practices on the seedling density. Seed germination tests with different water potentials, temperatures, and soil and water depths showed the environmental effects on the characters related to seed germination in CMV. Imbibition under different temperatures reflects that initial velocity was rapid at higher temperature, however, the times to full imbibition were not different between 15 and 25°C. The optimal germination temperature for CMV germination was ranged from 15 to 20 and the germination was highly affected by water potential of media at relatively high temperature above 20°C. When the seeds were sown in flooded condition the germination was not proportionally affected by water depth. In addition, there was no correlation between water depth and oxygen concentration. The germination of seeds flooded by 2 cm water depth were poorly germinated compared to other depths. Results indicated that the germination of submerged seeds was more highly influenced by flooding depth than the temperature, it was also affected more strongly at 10 than 20°C. Emergence of CMV depending on the thickness of covered soil was poor when the soil layer was greater than 5 cm. In the experiment with seeds collected between 22 days after flowering (DAF) and 52 DAF, the highest germination ability of CMV seeds was observed at 39 DAF and germinability was decreased subsequently as seeds became mature. The lower germinability may be due to the enhanced seed dormancy.

Keywords **:** *Astragalus sinicus*, Germination, Water potential, Emergence, Self-reseeding

In recent, Chinese milk vetch (*Astragalus sinicus* L.; CMV) have been reexamined to be used as an winter cover crop in upland and/or paddy fields due to its several advantages such as N-fixing ability, persisting greenness over winter season, and smothering activity against weeds in spring (Kim *et al.*, 2001; Jeong *et al.*, 1995; Yasue, 1991). The history of CMV cultivation in Korea peninsula reflects that this plant had been adapted in the region from long time ago. It was reported that the N-fixing activity of CMV was 150 to 200 kg $ha^{-1}yr^{-1}$ depending on the edaphic and/or environmental conditions and the biomass production was approximately 30,000-40,000 kg ha^{-1} per year. In addition, CMV is spontaneously occurred in most fields in Korea although it has been known that CMV is occurring prevalently in the southern part of Korea. In fact we have found natural habitats of CMV in high latitudinal regions of Korea, Gyeonggi province. The occurrence of CMV in high latitudinal regions suggests that CMV can be used as cover crop in whole country. CMV is winter biennial which has relatively higher low temperature tolerance. In fields, the most critical factor which directly related to the establishment of CMV is the germination ability after seed-shattering in fall. The germination of CMV seed, however, is uneven in fields because the shattering time is highly dependent on the sowing or transplanting time of following main crop. The reason showing different seed germination at each seed developmental stage may due to the level of seed maturity, hormonal level, and various environmental conditions like soil water status, temperature, and partial oxygen pressure in soil (Gay *et al.*, 1991; Pekrun *et al.*, 1997; Spanarkel & Drew, 2002; VanToai *et al.*, 1988).

In this study, we examined the effects of water status of media and temperature on the germination of CMV seeds. Germination is a physiological process by which growth is commenced from embryo. The process starts with imbibition and ends with the emergence of the radicle. Therefore, the germination of seeds sown into soil is affected by the level of soil water potential. The hydration level of seed required for proper germination is specifically dependent on a species. In order to know the optimal moisture condition of a species, germination response to the levels of water potential should be examined using artificially-controlled moisture conditions. Field emergence is the subsequent event after germination, which affected by soil depth and soil texture (Benvenuti, 2003). The soil depth and texture influence the oxygen concentration which affects the seed germination in soil.

Until now most results related to CMV have been focused on the residual effects of CMV on following crops, especially on rice plant. The self-reseeding of CMV at following year, however, is important because the cost of repeated

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sowing of CMV seeds is not negligible in the use of CMV as cover crop for environmentally friendly agriculture. The basic research on the habit of seed germination of CMV under various environmental conditions is rare. Therefore, we had conducted the germination-related experiments with different regimes of temperature and water status for improving the germination ability of CMV.

MATERIALS AND METHODS

Seed materials

Seeds of CMV were provided by Seedbank for Wild Herbaceous Species of Korea University.

Water imbibition test

The kinetics of water uptake of seeds over imbibition period under various temperatures was examined with distilled water. Seeds were soaked in petri dish and placed in incubator set at 15 and 25°C. Fresh weight of imbibed seeds was measured after removing the surface water with paper towel every 3 hr for 2 days.

Germination test

Seed germination test was carried out using an incubator with different temperature conditions. One hundred seeds were placed on a pertri dish lined with filter paper (Toyo No. 1). Distilled water was supplied on a petri dish whenever the water shortage was observed. To know the influence of water depth on the germination with respect to the germination property under hypoxia, seeds were placed on the bottom of 500 mL beaker with various water depths. Oxygen concentration in germination media was measured at the bottom of beaker by an oxygen electrode and compared to the values calculated by the formula for dissolved oxygen concentration (Truesdale & Downing, 1954). The experiment for knowing water depth effect was conducted in an incubator set at 15°C that had proven as the optimal temperature for CMV seed germination. In order to know the effect of water potential of media on the CMV germination water potential of medium was adjusted with polyethylene glycol (PEG 6000) based on the equation suggested by Michel & Kaufmann (1973). The equation was as follow: water potential (Ψ) = $-(1.18\times10^{-2})\ C-(1.18\times10^{-4})\ C_2+(2.67\times10^{-4})\ CT+(8.39\times10^{-7})\ C^2T$, where C is the concentration (g kg^{-1} H_2O) of PEG 6000 and T is the temperature (°C).

To know the optimal temperature for CMV seed germination itself, not due to the difference in water uptake rate, seeds were imbibed at 25°C for 12 hr which is the period enough to uptake water necessary for germination and place to incubators with different temperatures.

Emergence test

Emergence of CMV seedling in soil was tested in a greenhouse. Average temperature of the greenhouse was 25/18°C (day/night) during experiment. Fifty seeds were sown on a plastic pot (8 cm, inner diameter) packed with mixed soil (silty loam : peat moss = 3 : 1, v/v). Pots were placed on a plastic tray of 15 cm height. Irrigation frequency and the level of water table were controlled to impose the different soil moisture condition. Flooding treatment was conducted with seeds sown with 0.3 cm soil covering. Emergence test was performed with shattered seeds and unshattered seeds (contained in intact pods).

Seed composition

Contents of crude protein and reducing sugars during seed development in pods were measured at each sampling date with 1 week interval. Crude protein content was determined by Kjeldahl method (A.A.C.C. 1969). Reducing sugars were determined by DNS method and calculated over the standard curve with glucose (Bernfeld, 1955).

RESULTS AND DISCUSSION

Seed germination begins with imbibition. In CMV the imbibition kinetics was measured at 15 and 25°C (Fig. 1). Because the internal water potentials of seeds are same in sample seeds, the velocity of water uptake may dependent on the temperature. The result shows that initial uptake velocity was different between 15 and 25°C, however, there

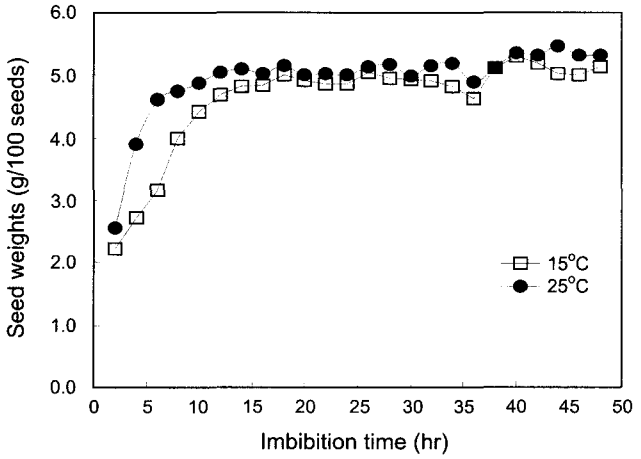

Fig. 1. Time course of water imbibition of CMV seeds at two different temperatures, 15 and 25°C. Seeds weight was determined after removing surface water with paper towel.

was no difference after 16 hr after imbibition. This result suggested that the relatively low temperature (15°C) was not a critical constraint to the imbibition for germination in CMV. The procedure after imbibition is the degradation of reserved materials in seed. As shown in Fig. 2, there was no difference in seed germination between temperatures above 15°C. On the contrary, the germinations at low temperature, below 10°C, were lagged (10°C) or lowered (0.5 and 5°C). Overall tendency implies that CMV can germinate appropriately when the seeds were placed above 10°C. In addition, it

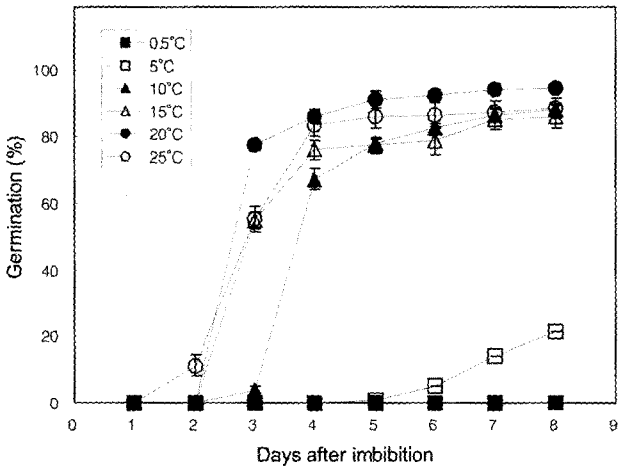

Fig. 2. Germination of CMV seeds at different temperatures.

can be assumed that proper emergence in CMV can be attained when the soil temperature is higher than 10°C. Besides temperature, water potential is also important factor in seed germination. We tested the water potential effect on the CMV germination. Under different water potential, ranging 0 to -1.5, CMV seeds were germinated differently (Fig. 3). Interaction between temperature and water potential on seed germination was reported by several researchers (Özbingöl *et al.*, 1998; Seong *et al.*, 1986). Water potential effect on the germination was conspicuous in the higher temperatures (20 and 25°C). The optimal temperature under 0 bar was 20°C, however, the overall optimal temperature was 15°C. This result implies that water imbibition at high water potential (0 and -0.5 bar) is may be more rapid than at lower water potential (-1 and -1.5 bar). Following events including internal metabolisms for degrading reserved materials and synthesizing new tissues such as shoot and roots after imbibition in CMV seed may be vigorous at 15°C. Because the overall procedure of seed germination includes imbibition and subsequent metabolic process for degrading reserving materials, apparent germination rate was higher at 20°C. At the water potential of -1.0 and -1.5 MPa the drastic decreases of germination were observed at 20 and 25°C, respectively. This result means that the drought stress is more critical when the CMV seeds were sown early. CMV seeds were sown before rice harvesting in paddy fields in which water status is not dry, in

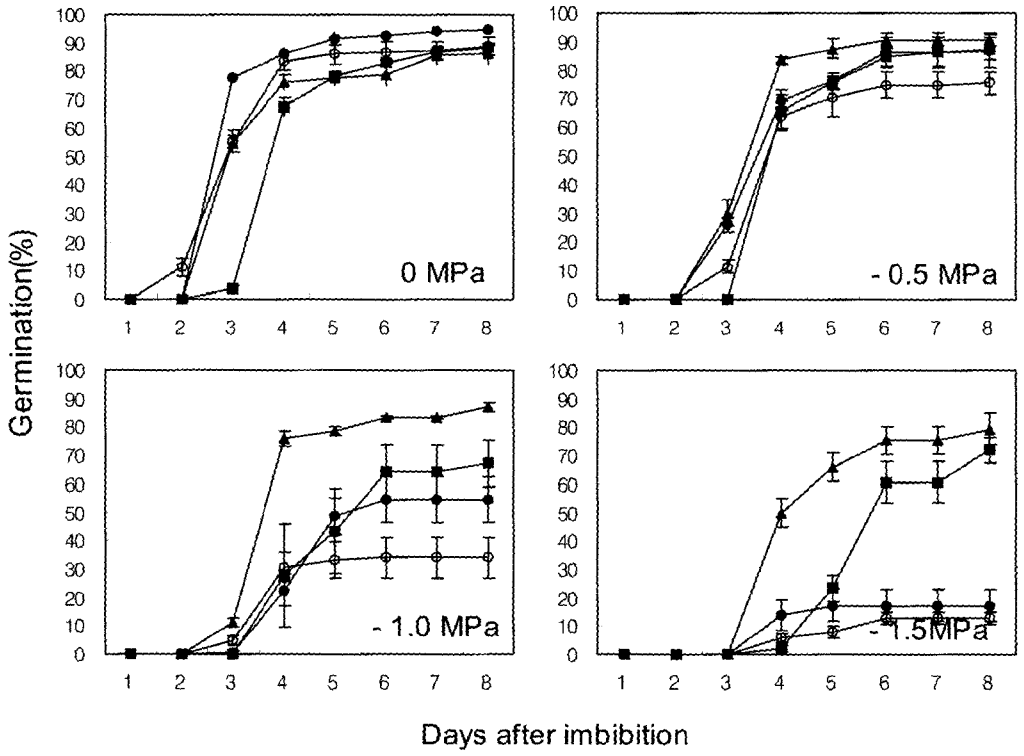

Fig. 3. Effects of temperature and water potential of media on the germination of CMV seeds. Water potential was controlled using PEG 6000 solution. Filled squares (■), filled triangles (▲), filled circles (●), and empty circles (○) indicate 10, 15, 20, 25°C, respectively.

upland fields, however, the soil moisture is sometimes low enough to inhibit CMV germination. Thus, CMV should be sown late when upland fields are subjected to drought.

Oxygen concentration influences on the germination and seedling growth (Aceves *et al.* 1975; Benveuti & Macchia, 1995). Here, we tested the water depth effect on the germination to know whether the oxygen concentrations changed by water depth directly affect germination. The oxygen concentration was measured on the bottom of beaker. The oxygen concentration of each water depth was presented in Table 1. There was no clear trend in oxygen concentration by water depth. When the seeds were submerged, the germination was not proportionally affected by water depth (Fig. 4). In addition, there was no correlation between water depth and oxygen concentration. Seeds submerged into water of 8 cm depth showed the highest germination percentage between flooding treatments. The seeds flooded with 2 cm water depth were poor in germination compared to other depths. Wuebker *et al.* (2001) reported that the effect of flooding on the germination of flooded seed is different at different temperature. In this experiment germination of submerged seeds was influenced by flooding more strongly at 10 than 20°C.

Table 1. Oxygen concentration at different water depths at 15°C and 25°C.

Water depth (mm)	Oxygen concentration (ppm)	
	15°C	25°C
Air saturated†	9.76	8.11
20	4.85±0.50	5.37±0.30
40	5.18±0.61	5.04±0.61
60	4.62±0.50	4.77±0.45
80	4.75±0.79	4.84±0.23

†Oxygen content of air saturated water was calculated by following formula (Truesdale & Downing, 1954). $Cs=14.16-0.39443T+0.007714T^2-0.0000646T^3$, where Cs=saturation concentration (ppm), and T=temperature (°C).

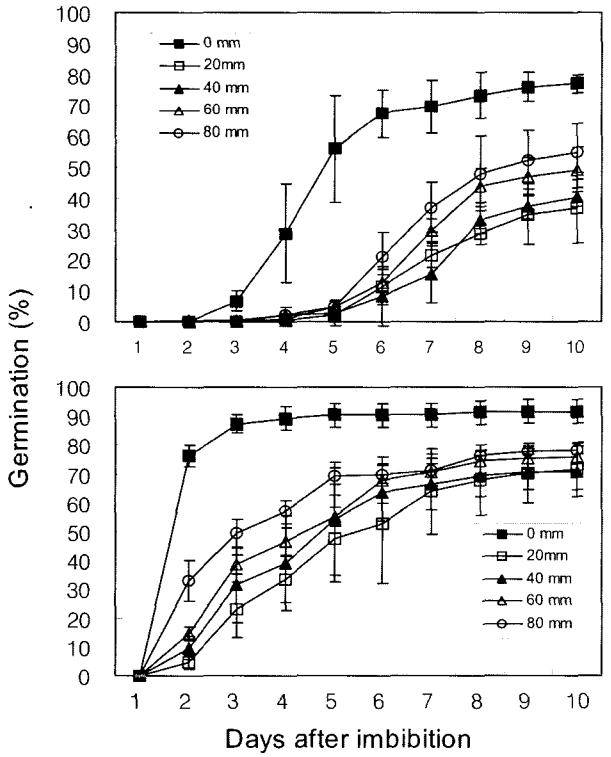

Fig. 4. Effect of water depth on the germination of CMV seeds at different temperature. Seeds were placed on the bottom of beaker. Upper and lower graph indicate 10°C and 20°C of water temperature, respectively.

Emergence of CMV in field condition in which covered soil depth and moisture condition was various was in part different from the germination in petri dish. Field emergence was highly dependent on the depth of covered soil (Fig. 5). Emergence was dramatically decreased as the covering layer increased above 8 cm. There were no significant differences in emergence when CMV seeds were sown with soil covering from 0.3 to 3 cm. The layer of 5 cm slightly decreased emergence, the influence, however, was not too high to show poor seedling establishment. When the seeds were placed under flooding condition after sown the emergence was lowered as the flooding depth increases (Fig. 6). The flooding depth higher than 5 cm strongly inhibited emergence. As shown in Fig. 4 the germination was highly affected by water depth in beaker. Although the field germination also decreased with deep water condition, however, lower water depth (less than 1 cm) did not reduce emergence. This result means that CMV should germinate properly under the water saturated soil condition by flooding with shallow water. Seedling growth under different soil depths and water depth was presented in Fig. 7 and 8, respectively. The highest seed-

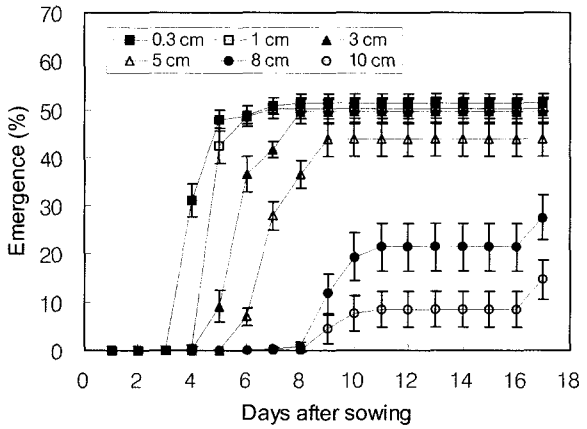

Fig. 5. Effect of the depth of covered soil layer on the emergence in CMV. Soil moisture condition was adjusted and maintained as field capacity with watering.

ling weight was observed when the seeds were cover with 1.0 cm layer. Seedling growth under thin covering (0.3 cm) was less than that under 1 cm soil covering, which may due to the shortage of moisture under thin soil covering. Dry weight of seedlings grown with thick covering (>5 cm) showed drastic decrease. This result suggests that the soil depth should be less than 5 cm. Seedling growth was decreased proportionally as the water depth increased. Seedlings grown in water deeper than 1 cm were highly inhibited in dry weight production. Because CMV is sown before rice harvesting in paddy fields, the sowing of CMV before the drainage of paddy water should be postponed until the paddy water was drained below 1 cm. Otherwise, the paddy water should be drained to promote the seedling establishment as soon as CMV was sown.

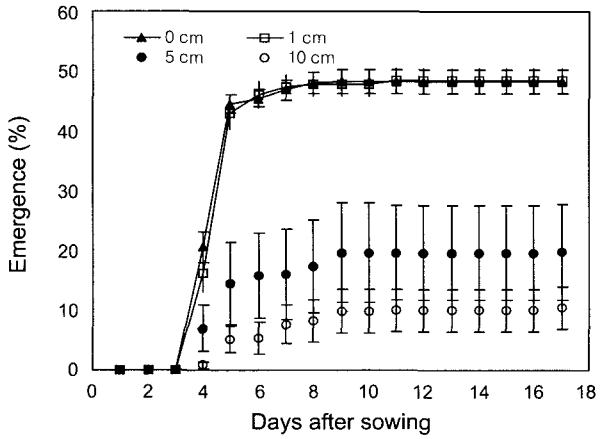

Fig. 6. Effect of the depth of flooded water on the emergence in CMV sown on soil with covering layer of 0.3 cm. Seeds were sown in pots and placed plastic tray flooded with different water levels.

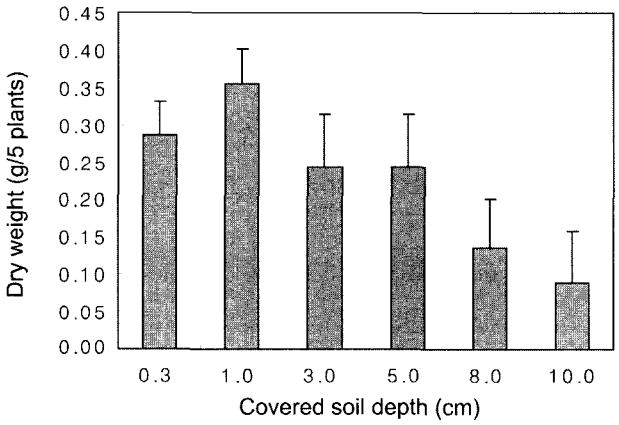

Fig. 7. Effect of the depth of covered soil on the CMV seedling growth. Soil moisture condition was maintained as field water capacity.

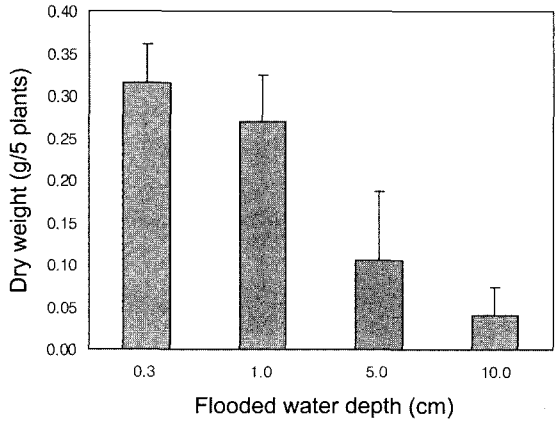

Fig. 8. Effect of flooded water depths on the CMV seedling growth. Seeds of CMV were sown with covering soil layer of 0.3 cm.

The purposes of the introduction of CMV into cropping system are to grow crop environmental-friendly and to save labor. For the purpose of labor-saving, the self-reseeding is the most important because repetitive sowing every year requires a lot of labor input. Therefore, farmers should establish the proper cropping schedule by which the self-reseeding of CMV is possible. The seeds collected from 22 DAF to 52 DAF were subjected to the test for germinability. The highest germination ability of CMV seeds was attained at 39 days after flowering at which total protein and reducing sugar contents became stable (Fig. 9 and 10) and then germinability decreased as seeds become matured. The lowered germinability may be due to the enhanced seed dormancy. The phenological approach suggested that CMV has a relatively higher germinability at 39 DAF and then the germinability decrease until the environment becomes to be favorable to germination. Difference in growth stage of a species is related to winter hardiness and tolerance to cold temperature (Fowler *et al.*, 1999). In practice the time for seed germination is important to attain proper CMV growth for promoting over-wintering capacity. In field, CMV germination can be observed during the growing period of rice,

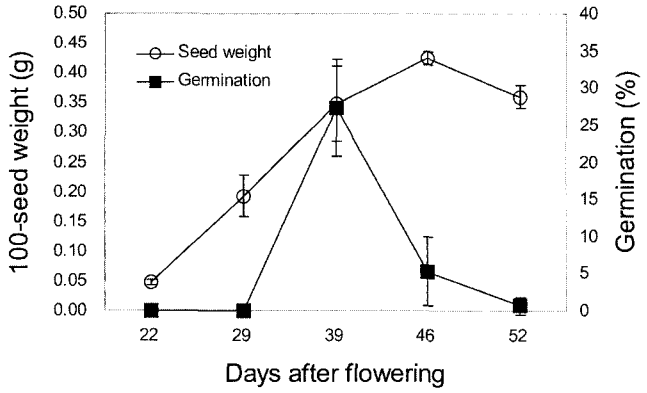

Fig. 9. Changes of seed dry weight and germinability of CMV seeds during seed developmental stage. Germination test was carried out with distilled water at 20°C as soon as pods were detached from plant.

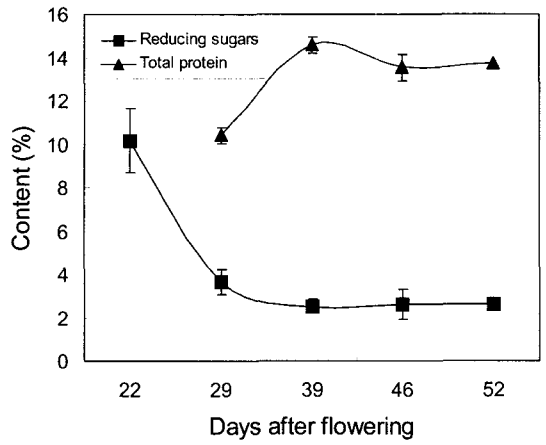

Fig. 10. Changes of the contents of reducing sugar and total protein according to the seed maturation in CMV.

however, germination initiation occurs in September at which rice plants are still growing. As shown above results the most optimal temperature for CMV germination is 15 to 20°C which is the temperature of September. Changes of seed composition reflected that the contents of protein and reducing sugar were stabilized at 39 days after flowering at which seed germination rate was the highest. Therefore, we can be assumed that the germinability was dependent on the physiological mature based on the chemical components of seed and the seed dormancy may triggered by the completion of the accumulation of chemical components in CMV seed.

In conclusion, the optimal temperature of CMV germination was between 15 to 20°C and the germination was highly affected by water potential of media under relatively high temperature above 20°C. Emergence of CMV, dependent on the thickness of covered soil, was poor when the soil layer was greater than 5 cm. There was no significant difference in the germination of submerged seeds between the water depth, however, the emergence of CMV was greatly decreased under flooded condition, which means that field-emergence would be poor under deep irrigated condition. Seed was ready to germinate at 39 days after flowering at which the germinability was the highest and then the germinability declined due to seed dormancy until the environmental condition become to be favorable temperature around 15°C. Considering the phenology of seed germination in CMV, water level in September in rice fields should be lowered below 1 cm to accelerate CMV emergence and growth before winter.

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