

Physical Dormancy in Seeds of Chinese Milk Vetch (*Astragalus sinicus* L.) from Korea

Sang-Yeol Kim[†], Seong-Hwan Oh, Woon-Ha Hwang, Sang-Min Kim, Kyung-Jin Choi, and Hang-Won Kang

Rice Ecology and Breeding Division, Yeongnam Agricultural Research Institute, NICS, RDA, Milyang 627-803, Korea

ABSTRACT Freshly harvested seed of Chinese milk vetch (*Astragalus sinicus* L.; CMV) was strongly dormant because of hardseedness. Seeds of freshly harvested germinated only 8% while clipping the seed coat completely overcome the innate dormancy, which indicates inhibition of germination of the seed is mainly due to seed coat (87%). The dormant (intact) hard seeds did not imbibe water whereas the non-dormant (clipped) seeds took up rapidly. In natural environment condition, the hard seed coat dormancy was broken only after 5 months after seed harvest. To break such a strong seed coat dormancy, the chemical and heat treatments were effective. Concentrated sulfuric acid was more effective than dry heat and hot water treatments. Hot water treatment improved germination but the germination percentage was less than 41%. Treatments increased germination due to its effect on the seed coat integrity. A scanning electron microscope revealed that disruption of seed coat layers and subsequent development of numerous crack in the hilum region of the seed and on the seed coat surface of concentrated sulfuric acid treatment and formation of cracks in the dry heat treatments, respectively, were observed in the seed coat surface, which served as water entry points.

Keywords : *Astragalus sinicus*, physical dormancy, scarification, water imbibition

CMV is a winter annual legume that has become an important green manure for sustainable production systems in southern Korea. The benefits included more profitable rice production milk vetch weed suppression, reduced fertilizer inputs and soil conservation (Jeong *et al.*, 1995; Jeong *et al.*, 1996).

Currently, CMV seed is totally imported from Democratic Peoples Republic of China but our own CMV seed pro-

duction is necessary because of concern of the introduction of foreign pest, disease and weed seeds which are a barrier of the sustainable rice production in Korea. In Korea, CMV seeds are generally harvested in the end of May and sow the seed in late September before final water drainage in rice field. However, freshly harvested CMV seed was reported to be highly dormant because of the hard seed coat (Suetsugu and Ueki, 1960; Shim *et al.*, 2004). This poor seed germination led to development of breaking seed dormancy to utilize the year harvest seed.

Methods of breaking hard seed coat dormancy of freshly harvested CMV seed must be developed to provide information on consistent establishment of CMV seedling stand during initial phase since germination ability is most critical factor in the seedling establishment in rice field.

Various artificial treatments have been used successfully to induce germination of CMV and seeds of many other weed species. These treatments include concentrated sulfuric acid (Suetsugu and Ueki, 1960; Robert *et al.*, 1984; Amin, 1987; Kim *et al.*, 1990; Martin and Curadra, 2004), dry heat (Walker & Evenson, 1985), and hot water (Baskin *et al.*, 2004). The effect of these treatments was different depending on the species. It has been well established that all these methods affect the integrity of seed coat. An understanding of dormancy mechanism of CMV seeds may help to develop strategies of seedling establishment in rice field. The objectives of this study were to investigate effective method for breaking hard seed dormancy of CMV seeds and to gain information on their mode of action.

MATERIALS AND METHODS

Seed sources and germination trials

Mature pod of CMV was hand-picked in May 30, 2007,

[†]Corresponding author: (Phone) +82-55-350-1174
(E-mail) kimsy3@rda.go.kr <Received September 23, 2008>

at the experimental field of Yeongnam Agricultural Research Institute, Milyang, Korea. Seeds were removed from pod manually by rubbing on a plastic net mat and stored in the 5°C refrigerator when seed not use.

Germination studies were conducted in a germinator maintained at 95% relative humidity and given continuous light at a constant 20°C based on the previous experiments. All germination tests were done using 9 cm petri-dishes lined with Whatman #1 filter paper. One hundred seeds were placed on the petri dish and 5 ml of deionized water was added. A completely randomized design was used with treatments replicated four times. Germination counts were taken at 2-day intervals over a 10-day incubation period. Seeds were considered germination when emerged radicle was 2 mm long.

To observe how each treatment improves dormant seed germination, changes in the surface of seed coats before and after scarification were observed using the scanning electron microscope (Hitachi S2460N and S3000N) at 20 Kev.

Effect of seed coat clipping on the seed dormancy break

Two seed treatments, intact and seed coat clipped, were evaluated for CMV seed dormancy. Small portion of the seed coat clipping was made manually with a sharp blade and care was taken to avoid damaging cotyledon. Germination was recorded at 1, 2, 3, 6 and 10 days after seed placement.

Water imbibition

One hundred manual clipped and intact seeds were placed on filter paper, moistened with 5 ml deionized water in petri-dish. After 6, 12, 24, 36 and 48 hr, the seeds were removed from the petri-dish and the surface of the seeds was blotted dry. The moisture content was determined by drying the seeds of 100°C for 72 hr. The moisture content was measured based on the dry weight. Seeds were observed for germination and final germinations were made after 72 hr.

Duration of physical dormancy

The freshly harvested seeds in June, 2006 were placed in the plastic net bags and stored in cage house. The cage house stored samples were drawn at 0, 30, 60, 90, 120, 150, 180, 210 days and tested for germination. At each germination

test, germinated seeds and the hard seeds were recorded. The hard seeds were judged from the seed which seed coat is hard and do not imbibe water.

Treatments for breaking physical dormancy

Concentrated sulfuric acid. All seeds were soaked in concentrated sulfuric acid (95-97% assay) for 0 to 30 min at 5 min interval. Seeds were steeped in a 100-ml glass beaker containing concentrated sulfuric acid. The volume of seeds and acid was maintained at 1:5 ratio. Seeds were then immediately placed on strainers, rinsed with a steady stream of tap water and finally with distilled water, then air-dried for one day before germination test was conducted.

Dry heat. The 20×15 cm plastic net bags contained 20 g seeds were placed in electrically heated convection type incubator at 70, 80 and 90°C for 1, 2, 3, 4, 5, 6 and 7 days. The germination test was conducted one day after treatment.

Hot water. Twenty gram seeds were placed in 20×15 cm plastic net bags. The bags were allotted random numbers and seeds were scarified by hot water at 70, 80 and 90°C. After scarification, seed bags were removed at 0, 10, 20, 30 and 40 min intervals and air-dried overnight before germination test was conducted.

RESULTS AND DISCUSSION

Physical dormancy break

The non-dormant (clipped) seeds started to germinate between 12 and 24 hr and sharply increased as time passed by (Fig. 1). Then, germination percentage of the non-dormant seeds was achieved nearly 100% at 2 d incubation. In contrast, less than 8% of the seeds germinated in the dormant (intact) seeds even after 10 d.

This result confirms that freshly harvested CMV seed is strongly dormant because of hard seed coat but not the immature of embryo development since freshly harvested intact seed germinated only 8% while clipping the seed coat completely overcome the innate dormancy. Thus, seeds of *A. snicus* from Korea have physical dormancy only, and not physical + physiological (combinational) dormancy, as has been reported for Rhamnaceae (Turner *et al.*, 2005) and

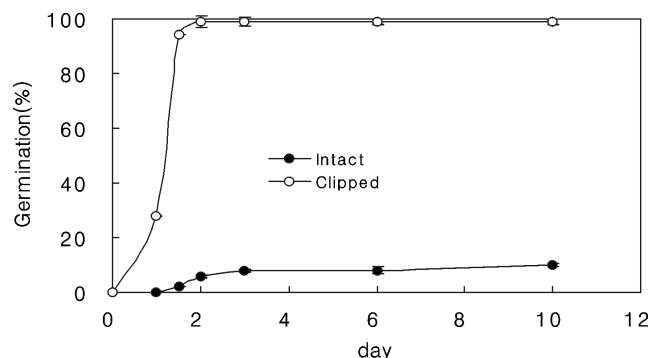


Fig. 1. Time course of germination in dormant (intact) and non-dormant (seed coat clipped) Chinese milk vetch seeds.

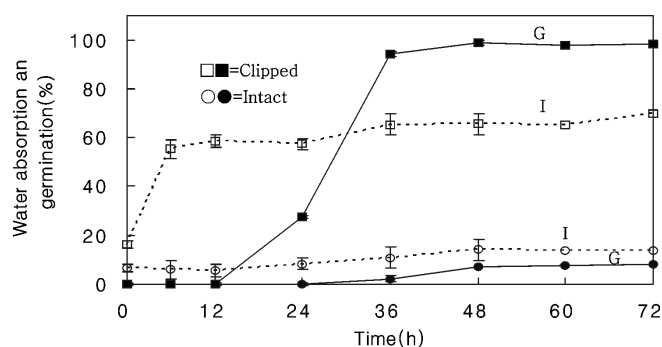


Fig. 2. Water absorption and germination patterns in dormant (intact) and non-dormant (seed coat clipped) seeds of Chinese milk vetch. G: germination, I: imbibition.

Dodonaea viscosa (Jerry *et al.*, 2004) wild okra (Velempini *et al.*, 2003).

Water absorption

Water absorption of clipped (non-dormant) and intact (dormant) seeds differed significantly within the first 6 hr of incubation (Fig. 2). Within this period, the water content of both non-dormant seeds increased sharply from a initial 16% to 55%. After 6 hr of incubation, the non-dormant seeds imbibed water slowly up to 24 hr and then slightly increased thereafter. The non-dormant seeds started to germinated between 12 to 24 hr when water content of 58% was attained. Seeds achieved nearly 100% germination by 48 hr. The increase in germination paralleled a rapid increase in water content. This confirmed that embryo development is not a limiting factor, rather dormancy appears to be associated with the presence of the covering layers as shown in Fig. 1.

In contrast, the dormant seeds imbibed only small amount

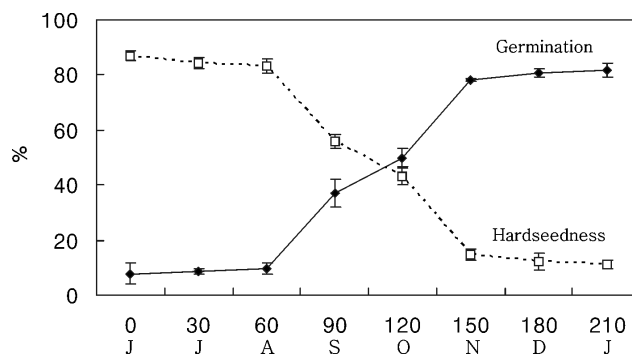


Fig. 3. Change of physical dormancy and hardseedness ratio of freshly harvested Chinese milk vetch seed as influenced by storage duration in cage house.

of water even after 6 hour of incubation, thereafter up to 72 hr of incubation, water absorption did not increase much. The dormant seed germinated after 48 hr of incubation, but water content was less than 14%. These results suggest that the seed coat of intact seeds of this species are clearly impermeable to water. This has been confirmed for several weed species (Werker, 1980). Patil and Andrew (1985) also found that hard seeded cotton (*Gossypium hirsutum*) cultivars absorbed significantly lesser moisture than their counterparts. Apparently non-dormant seeds imbibed water in amounts sufficient for germination whereas dormant seeds did not. Bewley and Black (1985) reported that preventing water entry to the embryo or limiting exchange of respiratory gases, and blocking light penetration to the embryo contribute to coat imposed dormancy in seeds.

Duration of physical dormancy

The high hard seed percentage of 87% was present in the freshly harvested CMV seeds and it was only decreased slightly up to 60 days after harvest (Fig. 3). However, after 3 months storage at the natural environment (September), the time of the CMV seed sowing in the rice field in Korea, the hardseedness was significantly decreased but it was still high with 58%, showing only 40% seed germination. Only after 5 months seed storage which is 60 days later than the seed planting date of late September in Korea, the hardseedness of CMV seed was decreased to 20% due to breakage of hard seed dormancy from 8% to 82%. This indicates that to plant CMV seed produced in Korea in rice field in the same year, the artificial pretreatment is necessary or use the

one year stored-seed which is generally broken dormancy naturally.

Treatments for breaking physical dormancy

Concentrated sulfuric acid

Immersion in a concentrated sulfuric acid for 5 min significantly improved the germination of CMV seeds and the effect was more pronounced with increasing the scarification time (Table, 1A). The seed reached the maximum germina-

Table 1. Germination of Chinese milk vetch seed treated with various durations in concentrated sulfuric acid (A), dry heat (B), and hot water (C).

(A)

Treatment (min.)	Germination (%)
0	8d
5	53c
10	80b
15	85b
20	93a
25	97a
30	94a

(B)

Treatment (day)	Germination (%)		
	70°C	80°C	90°C
0	8e	-	-
1	53d	57d	45bc
2	69c	75c	56a
3	78b	78bc	59a
4	79ab	82ab	50ab
5	85ab	86a	42bc
6	86ab	85a	35c
7	84ab	85a	20d

(C)

Treatment (min.)	Germination (%)		
	70°C	80°C	90°C
0	8f		
10	11f	28d	30cd
20	11f	32bcd	37ab
30	20e	33bc	41a
40	20e	30cd	39a

tion levels (93-97%) with soaking duration of 20 to 25 min.

Similar high germination of seed treated with concentrated sulfuric acid was obtained with CMV (Suetsugu and Ueki, 1960) and other species (Robert *et al.*, 1984; Amin, 1987; Kim *et al.*, 1990).

A scanning electron micrograph revealed no cracks in the hilum (Fig. 4A) and on the seed coat surface (Fig. 4B) of untreated seeds. However, increasing the scarification time with concentrated sulfuric acid dissolved seed coat layers and subsequently numerous cracks developed in the hilum region (Fig. 4C) on the seed coat (Fig. 4D). Creating points of water entry into the seed coat improved germination.

Similar changes in seed coat were obtained with *Opuntia* spp. (Robert *et al.*, 1984), *Sesbania rostrata* (Amin, 1987) and *Commelina benghalensis* (Kim *et al.*, 1990). This suggests that removal of water impermeable layers in the seed coat and subsequent development of cracks throughout the seed coat promoted water absorption, and thereby accelerated germination. Also, such disruption of the seed coat can induce other changes such as increased sensitivity of seed to light, temperature, and other factors (Khan and Samimy, 1982).

Optimum immersion time for scarification was found to be 20 to 25 min. When seeds were scarified for longer than 25 min, germination slightly decreased. Acid scarification at these durations probably damaged the embryos of some of the seeds thereby reducing germination.

Although concentrated sulfuric acid was very effective in breaking hard seed dormancy of CMV, it is potentially risky for seeds and operators and it is recommended only when no alternative methods are available.

Dry heat

Exposure to dry heat significantly increased seed germination in all temperatures but the effect was different depending on the treatment temperature and exposure time.

Exposure to 70 and 80°C for 5 d gave the maximum germination of 85-86% (Table 1B). Exposure to 90°C significantly increased germination but germination percentage was lower than that exposed to 70 and 80°C for 5 d. Walker and Evenson (1985) found that exposure to 75 to 90°C significantly reduced the impermeability of *C. benghalensis* seeds, irrespective of exposure time.

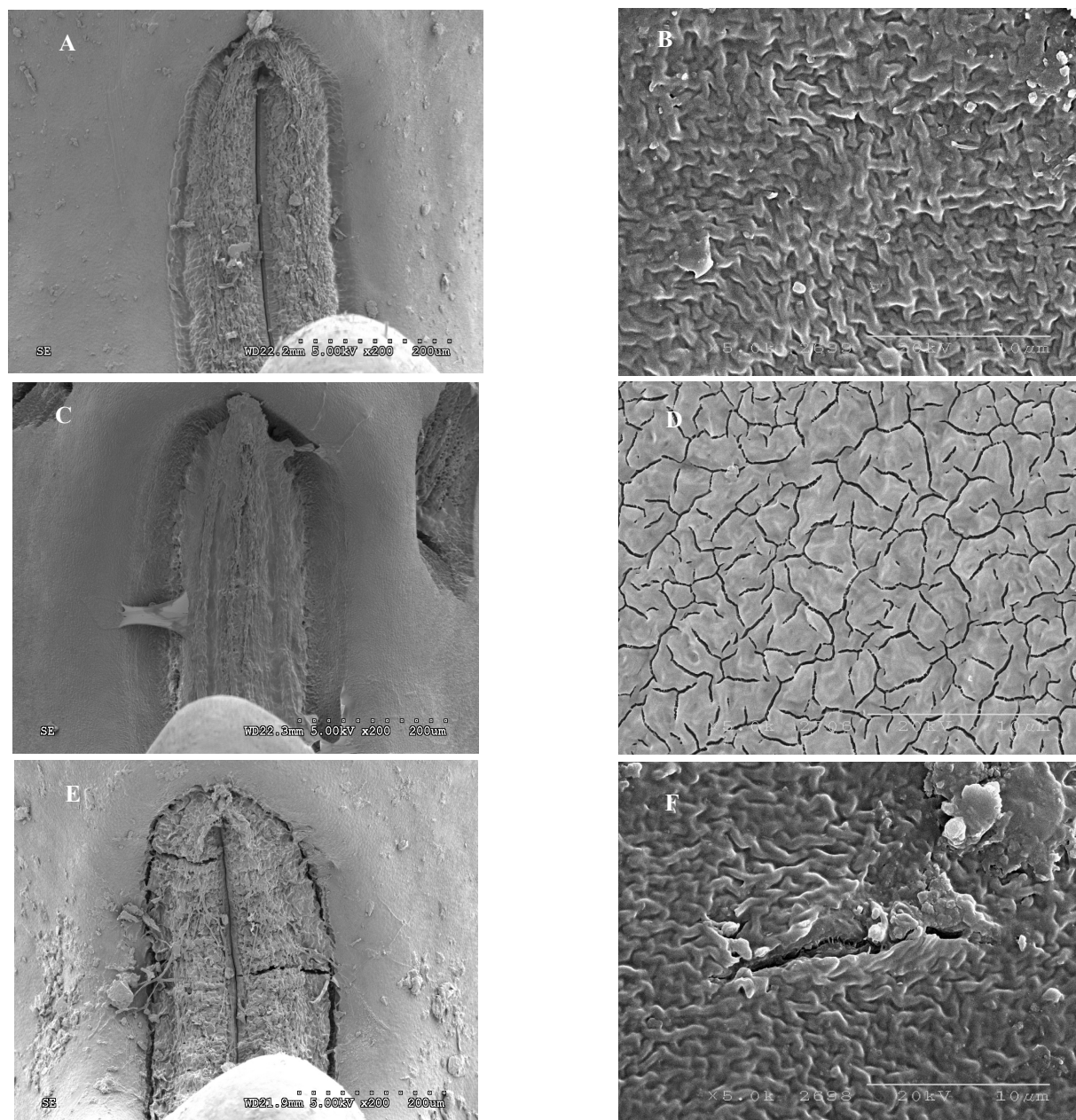


Fig. 4. Scanning electron micrograph of the hilum and seed coat surface of Chinese milk vetch treated with concentrated sulfuric acid (C, D) and dry heat (E, F), showing numerous cracks developed on the hilum and seed coat surface as compared with untreated control (A, B).

As with concentrated sulfuric acid treatment, dry heat treatment improved germination because of the subsequent formation of cracks on the hilum (Fig. 4E) and the seed coat surface (Fig. 4F), which served as water entry points. These cracks were probably caused by the differential thermal expansion in the structural elements of the seed coats.

Dry heat treatment at 80°C for 5d improved seed germi-

nation but total germination percentage was less than that with the concentrated sulfuric acid treatment.

Although the dry heat treatment was a time consuming and not as effective as that in the concentrated sulfuric acid, this method is advisable for being safe and easy method in breaking CMV seed dormancy at the farm level.

Hot water

Soaking seeds in hot water improved germination but its effect was negligible.

Germination percentage of seeds was slightly increased at 70°C hot water for 30 to 40 min and 80°C for 10 to 30 min but the germination percentage was less than 33% (Table 1C). On the other hand, germination was improved at 90°C for 10 to 30 min but the high abnormal seedling was observed due to heat damage (data not shown).

Results show that the dormancy mechanism of CMV seed is due to physical dormancy and it takes 6 months to break seed dormancy, which is far later than the seed planting date of late September in Korea. Although the dry heat treatment was a time consuming and not as effective as that in the concentrated sulfuric acid, this method is recommended as safe and easy method in breaking CMV seed dormancy at the farm level.

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