Changes of Seed Quality of Chinese Milk Vetch (*Astragalus sinicus* L.) During Seed Developmental Stages

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ABSTRACT The objective of this study was to investigate the variation of Chinese milk vetch (*Astragalus sinicus* L.; CMV) seed quality after flowering. We tagged individual open flowers of CMV at the day of maximum flowering (11 May) in Seoul, Korea. Seed samples were harvested serially at 15, 20, 25 and 30 days after flowering (DAF). To compare with dried seeds, non-dried seeds were tested immediately after harvest and the remaining seeds were placed at room temperature for 4 weeks. Seed length, 1000 seed weight, moisture content, germination rate (GR), mean germination time (MGT), germination speed (GS), germination performance index (GPI) and physical dormancy rate (PDR) were investigated. Seed length increased to 2.6 mm and 1000 seed weight reached up to 2.2 g until 25 DAF. Seed moisture content dramatically decreased from 20 to 25 DAF. Moisture content of non-dried seed (7.5%) was similar to that of dried seed (5.5%) at 25 DAF. The rate of seed viability reached up to 94% at 25 DAF. In case of dried seed, GR increased up to 39% at 25 DAF whereas GR of non-dried seed varied from 5 to 10%. GS and GPI of dried seed were significantly higher than those of non-dried seed since 25 DAF. PDR of dried seed has decreased since 20 DAF, whereas PDR of non-dried seed has increased. GR, GS and GPI increased as PDR decreased. Our results evidenced that PDR might be one of major factor in variation of seed quality, of which development was completed at 25 DAF.

Keywords : *Astragalus sinicus*, seed quality, seed development, days after flowering, drying treatment

Chinese milk vetch (*Astragalus sinicus* L.;CMV) is a biennial green manure crop belonging to the *Fabaceae* family, which is mainly cultivated in North-East Asia, e.g. Korea, Japan and China. In general, the ability to fix nitrogen of this plant leads to be used as a winter cover crop (Cho and Choe 1999). Also CMV is used to produce nectar for honeybees (Yasue 1986) and a pasture for livestock (Seong and Park 1991). Flavonoid compounds having potent antioxidative activity have been isolated from seeds of CMV (Yeom *et al.*, 2003).

CMV is known as an attractive green manure crop and has been encouraged to be adopted for sustainable rice production system in Korea. Because CMV seeds are entirely depended on import from China it is needed to research on the optimum harvest time and the physiological traits of seed for CMV seed self-support in Korea. A few of reports have been issued regarding seed germinability of CMV and natural reseeding. The germinability of CMV seeds evidenced a peak at 39 days after flowering (Shim and Kang 2004). Recently, Kim *et al.* (2007a) reported that natural reseeding technology of CMV showed some advantages as compared to seed-planting. The optimum time to incorporate CMV plants into soil for natural reseeding seems to be 40 DAF in the southern part of Korea (Kim *et al.*, 2007b). However, little is known on variation of CMV seed quality during seed development and effect of physical dormancy on the seed quality.

Seed harvest appears to be related to seed maturity and seed vigor. Vigor in the stage of preharvested seeds is low because of immaturity of an embryo or an endosperm and delayed harvest of seed makes that seed vigor is reduced by deterioration of seeds (Copeland and McDonald 2001). A few reports have been issued regarding degree of seed

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maturity at different collection time. The seeds of *Ulmus davidiana* var. *japonica* have to be collected just before drying morphologically according to the change of seed characteristics and germination properties at different seed collection time (Tak *et al.*, 2006). The germinability was investigated during the development and maturation of seeds of *Phaseolus vulgaris*, suggesting that normal germination rates from freshly-harvested to dried seeds were consistently increased during seed development (Sanhewe and Ellis 1996).

Seed-drying is known to play a role in the transition from a developmental program to a germinative mode, but not occurred at all development stage (Keromode and Bewley 1985, Kermode *et al.*, 1986). Immature seeds of several legume plants removed from their parent plant could not germinate on water unless first subjected to a drying treatment (Misra and Bewley 1985, Keromode and Bewley 1985, Kermode *et al.*, 1986). Seeds dried in pods removed from the plant for several days could continue to grow and develop within the pods through the detached pods and pod cylinders (Fountain *et al.*, 1989). Dormancy of CMV seeds was broken during seeds stored at room temperature after drying (Lee *et al.*, 2006).

Most of legume seeds showed a physical dormancy known as 'hardseededness' by seed coat impermeability, which inhibits water uptake (Baskin and Baskin 1998). This feature can be beneficial for survival in nature and increases probability of germinating when seeds are placed on adequately moist environment (Van Staden *et al.*, 1989). Therefore, all seed could not germinate at one time. Hard seed coat inhibits rapid and uniform germination (Plitmann and Kislev 1989).

Therefore, the objectives of this study were 1) to obtain basic data on the variation of seed quality according to seed development 2) to evaluate seed germinability in nature with dried seed after harvest 3) to test the change of hardseededness and the effect of hardseededness on the seed quality during seed development and 4) to estimate the optimum time for harvest and natural reseeding in the central area of Korea.

MATERICALS AND METHODS

Seed harvest and treatment

Seeds of CMV were harvested in 2006 at the Korea University, Anam-dong, Seongbuk-gu, Seoul, Korea. We tagged individual open flowers of CMV at the day of maximum flowering (11 May). Seed samples were serially harvested at each of 15 (25 May), 20 (30 May), 25 (5 June) and 30 (10 June) days after flowering (DAF). Non-dried seed samples were tested immediately after harvest. The remaining seeds were placed at room temperature (20-25℃) for 4 weeks to evaluate seed quality of dried seed. To estimate seed viability, seed scarification treatment was performed with sandpaper.

Physical characteristics of seeds

Seed characteristics were evaluated by seed length, 1000 seed weight and moisture content at different DAF. Seed length and 1000 seed weight were evaluated using only dried seeds and moisture content was evaluated using dried and non-dried seeds. Seed length was measured with three replicates of 10 seeds by an electronic caliper with 0.01 mm accuracy. 1000 seed weight was measured using three replicates of 50 seeds by a digital balance with 0.0001 g accuracy. Moisture content was determined on three replicates of 50 seeds. Seed samples weighed were placed in an oven at 80℃ for 48h and then reweighed.

Germination properties

Germination rate was evaluated by the standard germination test. The standard germination test was carried out using an incubator. Three replicates of 50 seeds were placed in an enclosure plastic cases (11×12×2 cm) lined with germination papers moistened with distilled water at an alternating temperature of 15/20℃ with light during 20℃ for 12h by a fluorescent lamp (four 40-W cool-white fluorescent lamp horizontally oriented on top of the incubator) (Lee *et al.*, 2006). Seeds were considered germinated when a normal root and a dicotyledon leaf structure were developed. Ungerminated seeds of scratched seeds were considered as dead seeds. The germination counts were made every other day for 30days.

Germination rate (GR), mean germination time (MGT), germination speed (GS), germination performance index (GPI) and physical dormancy rate (PDR) were calculated by germination counts data. GR was calculated as: GR = (N/S) × 100, where N is the total number of seeds germinated during germination test and S is the total number of seeds tested. MGT was defined by the equation: MGT = $\Sigma(t_i n_i)/N$, where t_i is the number of days from starting germination counts, n_i is the number of seeds germinated on day i and N is the total number of seed germinated during germination test. GS was calculated as: GS = $\Sigma(n_i / t_i)$, where t_i is the number of days from starting germination counts and n_i is the number of seeds germinated on day i. GPI was calculated by the equation: GPI = GR/MGT (Tak *et al.*, 2006). PDR was defined as: PDR=mean of non-germinated seeds/mean of viable seeds × 100.

Statistical analysis

This experiment was planed by nested design. The correlation coefficient (R) of individual data was evaluated by the Pearson correlation. The degree of correlation was determined as presented by Franzblau (1958). The significance of collection time on the each factor was determined by the *F* test. The Duncan's multiple range test at *P*-value < 0.05 was used for comparisons between the means of data. The difference between dried seed samples and non-dried seed samples at each harvest time was determined by the T-test. The statistical analysis was done using the SAS software version 8.02.

RESULTS AND DISCUSSION

An analysis of variance indicated a significant difference of viability, GR, GS and MGT on seed germinability test (P<0.05). The harvest date made a significant difference of viability, GR, MGT and GS. On the contrary, drying treatment at each harvest date was a significant factor in only GR and was not a significant source in other germination properties (Table 1).

There was a significant correlation between DAF and the seed physical characteristics in dried seed. DAF yielded not only a high positive correlation with seed length and 1000 seed weight (r = 0.939, r = 0.947) but also a marked negative correlation with moisture content (r = -0.601). Germination properties also had correlations with DAF and seed physical characteristics. DAF presented a marked correlation with GR and MGT (r = 0.785, r = 0.685) of germination properties. (Table 2).

In non-dried seed, moisture content showed a high negative correlation with DAF (r = -0.920) whereas DAF did not show correlations with germination properties. GR yielded a high positive correlation with GS (r = 0.939), had a marked correlation with MGT and GPI (r = 0.672, r = 0.698). GS showed a marked correlation with GPI (r = 0.783) (Table 3).

There were a few different results between dried seed and non-dried seed in correlation analysis. DAF affected on GR and MGT in dried seed, while DAF did not affect on germination properties in non-dried seed. Moisture content yielded a positive correlation with GS in non-dried seed but did not have correlation with germination pro-

Table 1. Analysis of variance of germination properties for drying treated Chinese milk vetch seeds at various Days after flowering

Effect	Germination properties				
	Viability	GR	MGT	GS	GPI
Model		***	*	*	ns
Days after flowering	***	*	**	**	ns
Drying treatment (DAF)		***	ns	ns	ns

*, ** and *** mean statistically significant at p<0.05, p<0.01 and p<0.001, respectively. ns indicates not significant DAF: days after flowering; GR: germination rate; MGT: mean germination time; GS: germination speed; GPI: germination performance index.

Table 2. Correlation coefficient between two variables in days after flowering, seed characteristics and germination properties of dried Chinese milk vetch seed samples.

	DAF	SL	MC	TSW	GR	MGT	GS
SL	0.939***						
MC	-0.601*	-0.590*					
TSW	0.947***	0.954***	-0.517				
GR	0.785**	0.653*	-0.374	0.723**			
MGT	0.685*	0.744**	-0.136	0.742**	0.533		
GS	0.240	0.101	-0.234	0.171	0.667*	0.048	
GPI	-0.141	-0.258	-0.277	-0.274	0.175	-0.604*	0.613*

*, ** and *** mean statistically significant at p<0.05, p<0.01 and p<0.001, respectively. DAF: days after flowering; SL: seed length; MC: moisture content; TSW: thousand seed weight; GR: germination rate; MGT: mean germination time; GS: germination speed; GPI: germination performance index.

Table 3. Correlation coefficient between two variables in days after flowering, seed characteristics and germination properties of non-dried Chinese milk vetch seed samples.

	DAF	MC	GR	MGT	GS
MC	-0.920***				
GR	-0.634	0.484			
MGT	-0.0516	0.013	0.672*		
GS	-0.462	0.598*	0.939***	0.464	
GPI	-0.538	0.548	0.698*	0.223	0.783**

*, ** and *** mean statistically significant at p<0.05, p<0.01 and p<0.001, respectively. DAF: days after flowering; MC: moisture content; GR: germination rate; MGT: mean germination time; GS: germination speed; GPI: germination performance index.

perties in dried seed. MGT had a negative correlation with GPI in dried seed but had a positive correlation with GR in non-dried seed.

There were significant effects of the harvest date on the seed length and 1000 seed weight (P<0.001). Seed length and 1000 seed weight were increased until 25 DAF but not changed after that time. At this stage, seed length was 2.6 mm and 1000 seed weight was 2.2 g. In non-dried seed, moisture content was dramatically decreased between 20 and 25 DAF but not changed after 25 DAF such as seed length and 1000 seed weight. Moisture content of non-dried seed (7.5%) was similar to dried seed (5.5%) since 25 DAF (Fig. 1).

Seed growth is accomplished by three phase. Seed moisture content decreases from 80% to 10% and seed weight increase through seed growth phase. In the last phase, seeds are dried and seed dry weight and chemical composition is not changed (Choi *et al.*, 2006). *Ricinus communis* and *Phaseolus vulgaris* seeds were not developed after drying because the mRNAs for developmental proteins were not produced (Bewley *et al.*, 1989). Soybean seed weight and size increased and moisture content decreased during development. After drying, physical characteristic and composition of soybean seed was not changed (Sale and Campbell 1980). CMV seeds used in this study yielded a similar tendency. At 25 DAF, seed drying occurred and seed physical characteristics were not changed any more. Both of 25 and 30 DAF had similar results on seed physical characteristics.

The rate of seed viability increased from 0% at 15 DAF to 94% at 25 DAF but not changed after that (Fig. 2). GR of dried seed increased at 20 DAF and reached a peak of 49% at 30 DAF. However, GR of non-dried seed did not show significant changes at all sampling times with a range

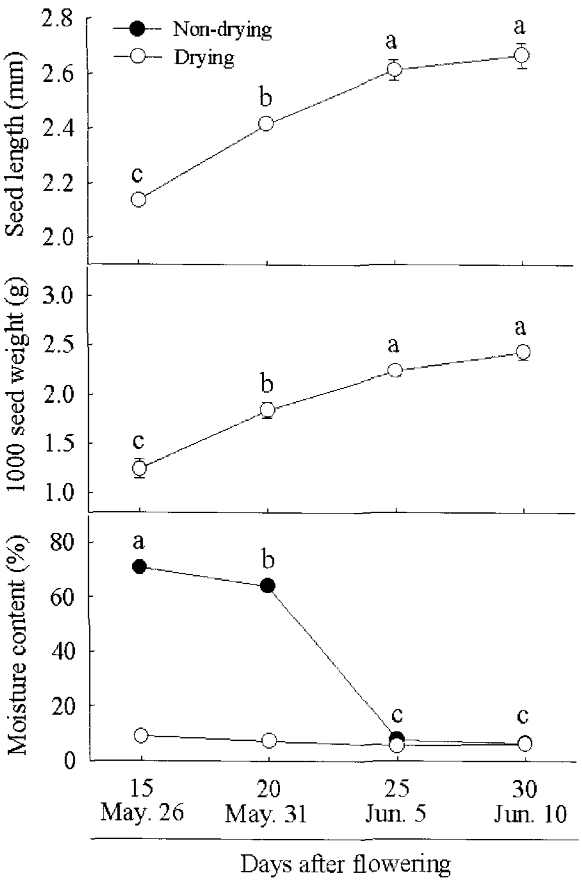

Fig. 1. Change of seed characteristics of Chinese milk vetch at various days after flowering. The different letters indicate significantly different at P<0.05 by Duncan's multiple range test. The vertical bar means ±1SE.

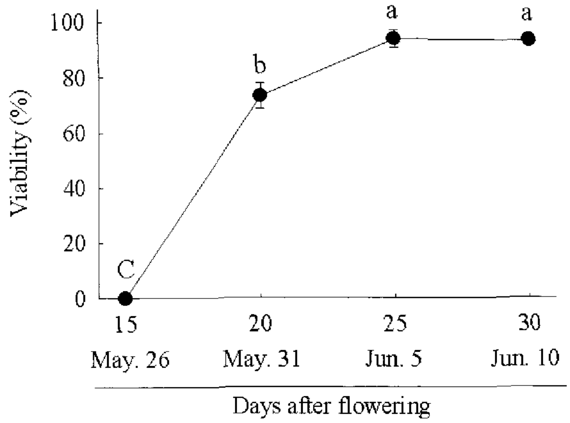

Fig. 2. Change of viability of Chinese milk vetch seed at various days after flowering. The different letters indicate statistical significance for the main effects of days after flowering. Viability from days after flowering with the same letter did not differ at P<0.05 according to Duncan's multiple range test. The vertical bar means ±1SE.

of 5 to 10%. GR of dried seed was significantly higher than non-dried seed at all sampling time except 15 DAF. MGT and GPI were not significant on the harvest time but GS of non-dried seed showed a significant difference on the harvest time. GS and GPI of dried seed were significantly higher than those of non-dried seed at 25 DAF and 30 DAF. PDR showed differences between non-dried seed and dried seed. PDR increased until 20 DAF in both dried and non-dried seed. However, PDR continuously decreased in dried seed and increased a little in non-dried seed after that time (Fig. 3).

In this study, PDR results can explain germination properties of CMV seeds. PDR of non-dried seed was higher than that of dried seed. High PDR means that germination is slower and uneven. Thus, GS and GPI of non-dried seed were lower than those of dried seed. At 15 DAF, seed samples were not likely to have physical dormancy. So, GS and GPI were high at 15 DAF. PDR of dried seed exhibited a peak at 20 DAF and subsequently decreased. Thus, GS and GPI of dried seed evidenced the lowest at 20 DAF and then increased. On the contrary, PDR of non-dried seed increased since 20 DAF, therefore GS and GPI decreased since 20 DAF.

Physical dormancy of CMV seed might affect on seed quality. When PDR is lower, seed quality was improved. Thus, dormancy breaking is important to usage of CMV seed. Dormancy of CMV seeds was broken during seeds stored at room temperature after drying. In addition, warm stratification was highly effective in breaking dormancy (Lee *et al.*, 2006).

In our study, development of CMV seed was continued until 25 DAF and seed quality was no longer improved since 25 DAF. Seed length, weight and moisture content did not change anymore since 25 DAF. Viability and GR of dried seed were not changed after 25 DAF.

However, Shim and Kang (2004) reported that CMV seed had the highest germinability at 39 DAF. This difference seems to be attributed by tagging time of flowering. In our report, DAF was started from the day of

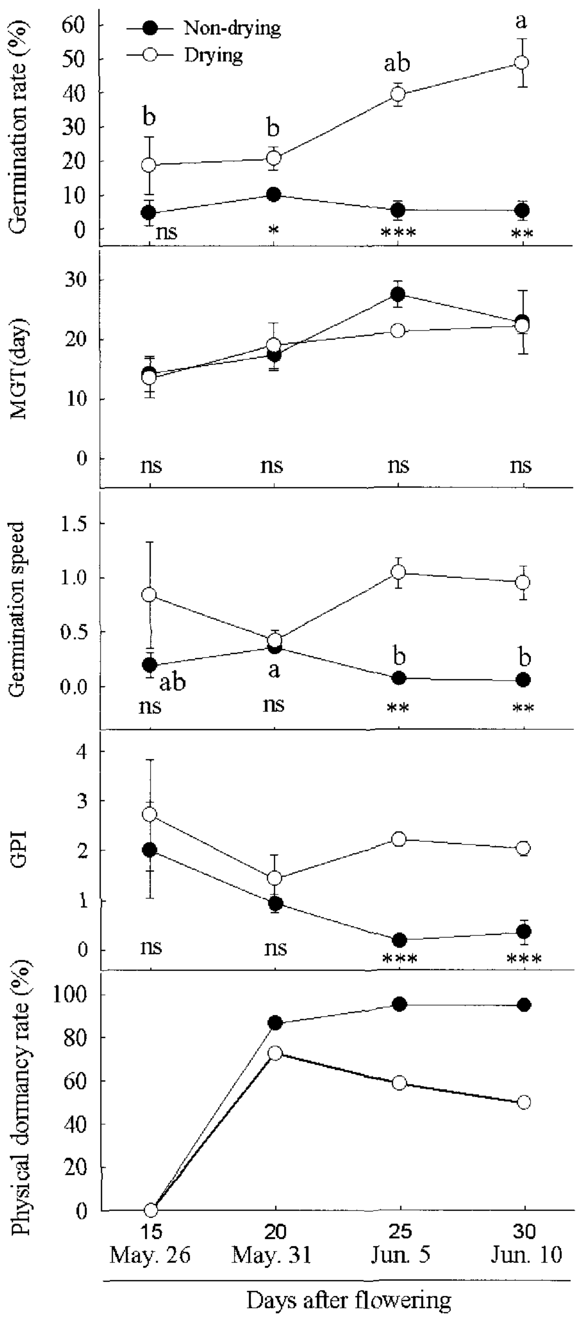

Fig. 3. Change of germination properties of Chinese milk vetch seed at various days after flowering. The different letters indicate statistical significance for the main effects of days after flowering. Germination properties from days after flowering with the same letter did not differ at $P<0.05$ according to Duncan's multiple range test. *, ** and *** indicate differences between dried seed samples and non-dried seed samples at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. ns indicates not significant The vertical bar means ±1SE. MGT: mean germination time; GPI: germination performance index.

maximum flowering whereas Shim and Kang (2004) set up DAF from time to first flowering. GR of dried seed at 25 DAF (39%) was similar to that at 30 DAF in the previous report by Shim and Kang (2004). Kim *et al.* (2007) reported that the optimum time to incorporate CMV plants into soil for natural reseeding seems to be after 30 May in the southern part of Korea. In our study, quality of CMV seed was completed at June 5 (25 DAF). Time of first flowering in the southern part is generally 2 weeks faster than that of the central part of Korea. However, the optimum time for natural reseeding in the southern part (30 May) was similar to the completion time of CMV seed quality in the central of Korea (5 June).

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