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Overwintering Capacity Affected by Seeding Time and Method of Chinese Milk Vetch, *Astragalus sinicus* L., in Upland Field

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ABSTRACT : Overwintering capacity, closely related to winter hardiness, of Chinese milk vetch planted with different sowing times and sowing practices was investigated to improve the incorporation into cropping system in Korea. The tolerance to low temperature was evaluated with LT_{50} using leaf disc leaching method. Dry weight of CMV was reduced remarkably with delayed planting from Sep. 5 to Oct. 20. The differences in tolerance to freezing temperature were not conspicuous among CMV genotypes, however, the differences between genotype (collections at different regions) were due to the plant architecture, mainly to the leaf angle. The crouching genotype collected at central region of Korean peninsula, which showed excellent freezing tolerant, has planophile leaves. The feature of internal constituents of CMV genotypes did not show any noticeable differences with respect to the freezing tolerance which evaluated by leaf disc leaching experiment. To overcome the poor overwintering capacity, tolerant genotype should be developed by selection with considering the plant architecture. The reduction of CMV growth during overwintering period was ameliorated with furrow-sowing under late-sown condition, therefore, when the CMV is inevitably sown late after recommended time, the seeds should be sown on furrow to overcome the cold stress.

Keywords: Chinese milk vetch, cover crop, overwintering, freezing tolerance, sowing practice

One of the prerequisites for developing environmental-friendly agriculture is to develop cover crop resources that have a function of green manure for reducing the application level of chemical fertilizer. A cover crop appropriate in Korean agricultural environment should be tolerant to low temperature during winter. The higher overwintering capacity can be observed in Chinese milk vetch, hairy vetch, and other perennial leguminous species like clover. Of the low temperature tolerant species, Chinese milk vetch and hairy vetch are currently grown in cultivated fields without noticeable growth retardation next spring. Chinese milk vetch (CMV) is a legume which exhibits relatively higher biological N-fixing activity (150 to 200 kg · ha^{-1} · yr^{-1}). In addition, the introduction of legume cover crop may helpful to suppress the occurrence of weeds in fields, therefore reduces the amount of applied herbicides (Brandsæter, 1999). Although CMV has agronomical benefits, the regional variation of overwintering ability has been known as a critical factor which obstacles to broaden the acreage of CMV-food crop cropping system.

There are several approaches to improve the winter hardiness which related to the overwintering ability. One of the ways to improve the overwintering capacity of CMV is to develop a strain that has a strong freezing tolerance. In fact we have found natural habitats of CMV in high latitudinal regions of Korea, Gyeonggi province. The spontaneous occurrence of CMV in high latitudinal regions suggests that CMV can be used as cover crop in whole country. CMV is a winter biennial which has relatively higher low temperature tolerance. The other way to increase the overwintering of winter crop is obtained with the modification of cultural practices like planting time and method. There are some practical methods to improve the overwintering ability like mulching with plastic film or straw, sowing on a furrow, and controlling the sowing time. The modification of sowing practice like furrow-sowing can be an alternative to increase the freezing tolerance because the seedlings on furrow can be protected from cold wind during overwintering.

To improve the amount of standing crop, the overwintering rate of CMV should be raised. The recommended timing of sowing ranges from mid-Sep. to late-Sep. depending on the cropping system and climatic condition. Therefore, in practice, CMV seeds are seeded before the rice harvesting to avoid the damage of freezing stress when the seedlings are too young to overcome the low temperature stress before overwinter.

Winter hardiness is an important trait to the overwintering plant. Freezing temperatures are a major factor determining the geographical locations suitable for growing crop and horticultural plants and periodically account for significant losses in plant productivity (Thomashow, 1999). In case of CMV, winter hardiness may dependent on the internal or external condition during winter. Internal conditions like the level of carbohydrates (Dionne *et al*, 2001; Dalmannsdottir

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et al, 2001; Alberdi & Corvuerra, 1991; Steponkus, 1984; Shahba *et al*, 2003), osmotica (Thomas & James, 1993), and composition of fatty acids (Cloutier & Siminovitch, 1982b) were studied by many researchers. An increase in freezing tolerance in herbaceous plants during cold acclimation is accompanied by a decrease in tissue water content (McIntire *et al*, 1987). In the case of white clover, starch content of stolon was higher in Dec., Jan., and Feb., reducing sugar content, however, lower in the period and higher in spring after overwinter (Turner & Pollock, 1998) reflecting cold hardiness of a variety is related to its ability to (1) reduce water content in tissues initially and (2) increase the amount of tightly and moderately bound water in the later stages. Varietal differences in hardiness may thus arise due to variations in both physical state and absolute amount of water (Yoshida *et al*, 1997).

External conditions like temperature and soil water content are varies according to year and related to the freezing tolerance (Cloutier & Siminovitch, 1982a). In addition, there are interactions between internal condition and external condition. Drought and freezing tolerance have been closely related because freezing is caused by the change of water to ice crystals in intercellular spaces of plant tissues (Steponkus, 1978; Siminovitch & Cloutier, 1983).

The success of CMV-involved cropping system in the northern regions of Korea should be determined by the development or breeding of cold hardiness CMV genotype. In winter, CMV open covered with snow by which CMV accumulate metabolites that may be responsible for membrane damage during ice-encasement stress (Pomeroy *et al*, 1983). Brandsæter *et al* (2002) reported that tolerance to low temperature in legumes which can be used as cover crop is dependent on the growth stage, however, the responses pattern at each stage to low temperature was different according to species. The differential response to low temperature may be due to the status of accumulated photosynthates and its translocation in plant tissues. Therefore, the low temperature tolerance is highly related to the nutritional condition of legumes.

The objectives of this study are 1) to obtain the basic information for improving the over wintering ability which has been known as a limiting factor to widen the growing area of Chinese milk vetch (CMV) in the middle region of Korea 2) to clarify the effect of sowing method on the over-wintering and growth of CMV plant and 3) to know the relationship between the level of internal substances and winter hardiness.

MATERIALS AND METHODS

Plant cultivation

CMV seeds were provided from Seedbank for Wild Herbaceous Plant, Korea University. Seeds were sown at Sep. 5 and Oct. 20 on experimental farms of Gyeongsang National University. No fertilization was conducted after harvest of preceding crops. Seeds were sown on the base of seeding furrows 10 cm deep and covered with soil of 2 cm after soil preparation or sown conventionally (levy-sown) without preparing seeding furrows. Any fertilizer and pesticide have not been applied during experiment.

Freezing tolerance test

Plants were tested for freezing tolerance in the laboratory and field. Each genotype was measured separately with three replications. Freezing injury was evaluated by leaf disc method using controlled circulating water bath. Electrolyte leakage was examined on the basis of the principles described by (Eugénia *et al*., 2003). In brief, tissue samples placed in screw cap tubes were subjected at different test temperatures in a low temperature circulating bath and controlled the temperature regimes by PC program (LabTracer, Jeiotech, Korea). After each freezing test temperature, samples were thawed at 4 °C for 2 h, and 20 mL of deionized water added to each tube. The tissue samples were held at 4 °C for 24 h and Electrolyte leakage was measured with an EC meter. After EC measurements were made on all treatments, all samples were boiled for 15 min and EC measured.

Plant analysis

Anthocyanins (Seigelman & Hendricks, 1958), Chlorophyll (Arnon, 1949), sucrose (Van Handel, 1968) and proline (Chinard, 1952) content in the leaves of CMV sampled with 1 week interval were determined using spectrophotometer based on the extinction coefficient (anthocyanins and chlorophyll) or standard curve (sucrose and proline).

RESULTS AND DISCUSSION

Innate freezing tolerance of CMV plants was examined by leaf disc method which used detached leaf segment from fully expanded leaves and subjected to a controlled bath set at test temperatures. Freezing tolerance of CMV genotypes in terms of the degree of amount of leachates was exhibited in Fig. 1 and Table 1. LT_{50} of CMV plant was various depending on genotypes. Tolerance of leaves from different positions also was different at mid-March (Table 1). The freezing tolerance was higher in young leaves from upper leaf-position than those from lower position. The relatively lower tolerance based on the LT_{50} of lower leaves may due to the lower level of cellular integration not to the metabolic activities linked to the synthesis of osmoprotectants. The

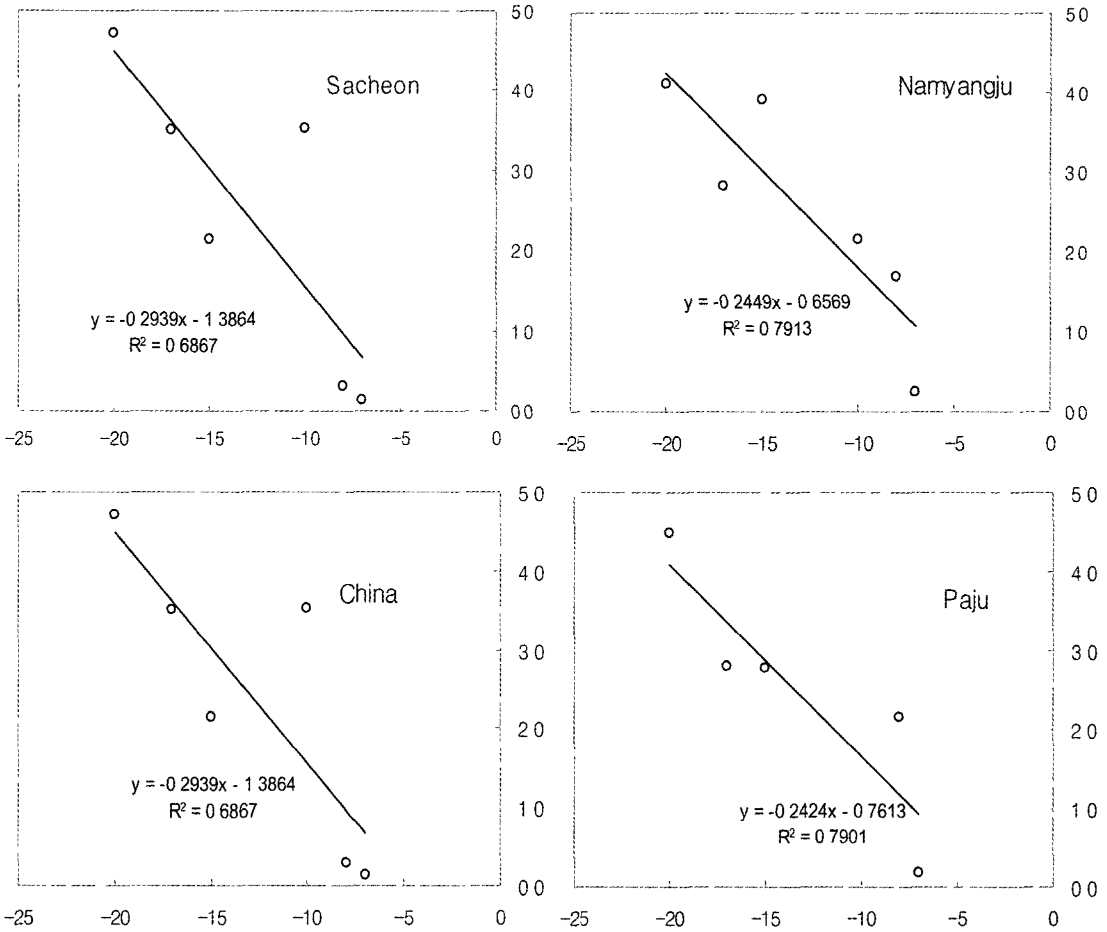

Fig. 1. Relationship between freezing temperature and solute leaching (logit) in upper leaves of CMV on Mar 20. X and Y axis indicate the treated temperature and logit response of leaf discs, respectively.

Table 1. Estimated LT_{50} of the leaves of CMV collection after overwinter (Mar. 20).

	Upper leaves	Middle leaves	Lower leaves
Sacheon	-10.02	-8.00	-6.46
Dukso	-6.77	-8.78	-8.45
China	-8.12	-6.17	-7.92
Wando	-7.69	-7.16	-7.02
Paju	-7 27	-7.82	-5 96

LT_{50} determined with leaching level of electrolytes did not show conspicuous difference between CMV collections (genotypes). The result from LT_{50} in CMV collections might reflect that internal mechanism for cold tolerance is less important than other factors like ecological, anatomical, and morphological traits in the ability to endow overall feature of cold tolerance because the degree of tolerance estimated from LT_{50} was not great and not enough to explain the genotypical difference between collections to the tolerance. The morphological data (Table 2), however, indicate big differences among CMV collections. An interesting trait of CMV from northern part of Korea was plant (canopy) type. The leaf angle of the plant from colder region was less than that of others. The leaf angle of Paju collection showed more prostrated canopy as compared to those from southern part of Korean peninsula. Plant height (shoot length) was greater in the CMV collections naturally growing in warmer region than those from cold region. The smaller leaf angle and height suggest that the morphological adaptation to cold region in which lower temperature is accompanying cold wind is more important to survive under cold temperature lower than physiologically critical temperature which can be estimated with LT_{50} parameter. In addition, a well-developed crown was observed in the CMV collections from higher latitudinal regions. The crown can supply a lot of reserved materials to shoot and leaves after overwinter and can be helpful to be established vigorously in spring season.

Morphological differences of CMV collections showed

Table 2. Growth characteristics of CMV overwintered in field condition.

Collections	Leaf angle (°)	Plant height	Shoot FW (g)	Shoot DW (g)	Moisture content (%)	Stem dia. (mm)	Crown thickness (mm)	Root FW (g)
Paju #1	63 8c	19.0f	9 30c	1.44c	84.0b	1.88d	6.62b	1.83b
Dukso	75.7a	31.8b	5.83d	0.77d	86.8a	2.11c	5.21d	0 45d
Wando	67.9b	25 4c	6.36d	1.05d	83 3b	2.16c	5.75c	0.67d
Paju #2	60 1d	22.1e	10.26c	1.67c	83.8b	2.09c	7 04b	1 34c
Sacheon	65.5bc	23.0d	17.30b	2.69b	84.4b	2 54a	8.50a	2.25a
China	73.2a	34 9a	24.82a	3.72a	85.0b	2.41b	8.51a	1.71b

Table 3. Comparisons of chemical components, shoot dry weight, and RWC between CMV collections on Feb. 23.

Collections	Anthocyanin (mg/g FW)	Chlorophyll (mg/g FW)	Sucrose (mg/g FW)	Reducing sugar (mg/g FW)	Proline (mg/g FW)	Dry weight (g/plant)	RWC
Paju	69.9±3.6	3.12±0.3	16 3±2.0	15 9±1.6	0.30±0.02	1.28±0 20	84.1±2.0
Dukso	77 9±4 6	3.01±0.08	18.1±1.8	17.3±1.4	0 32±0.03	0.89±0.06	84.2±1.5
Wando	65.8±1.4	3.34±0.19	16.9±0 1	19 3±1.4	0.46±0.02	1.14±0.08	79.8±0.4
Sacheon	90.4±3.2	3 17±0.06	25.7±1.9	20.1±1.0	0.22±0 01	3 41±0.36	87.5±3.6
China	82.1±6.2	3.76±.42	15.9±2.5	19.3±1.3	0.24±0.03	0.91±0.17	87.1±0.8

relatedness to the winter hardiness that endows survival in higher latitudinal regions in Korea. Because freezing tolerance directly affects to the growth and productivity of plant next spring (Thomashow, 1999), genotypes that have a morphology related to freezing tolerance should be planted to cold region in Korea. The leaf angle (inclination) of CMV collections from middle region of Korea was smaller than those from other regions. The smaller angle from soil surface means that the plant architecture is less erected and the creeping type which consisted of planophile leaves. In morphological feature, shoot to root ratio also showed difference between collections from different regions. The smaller shoot to root ratio might be originated from the well-developed root system and reflect that the plant with well-developed root system have a higher potential of winter hardiness.

The status of vegetative stage before winter varied with planting date in fall was also influential to winter hardiness. The result of planting date exhibited that the earlier the sowing that directly influence the size of aerial part, the greater tolerance to low temperature during winter. Because a plant with much more biomass can supply materials to reconstitute metabolites like osmoprotectants required to overcome the freezing tolerance. Although the data for metabolite was not significantly different between all genotype examined (Table 3) and no noticeable differences between tolerant (Paju) and less tolerant genotypes (China), the growth status controlled with planting date was closely related to the winter hardiness based on the dry weight after overwinter in CMV (Fig. 2). The dry weight of CMV after overwinter

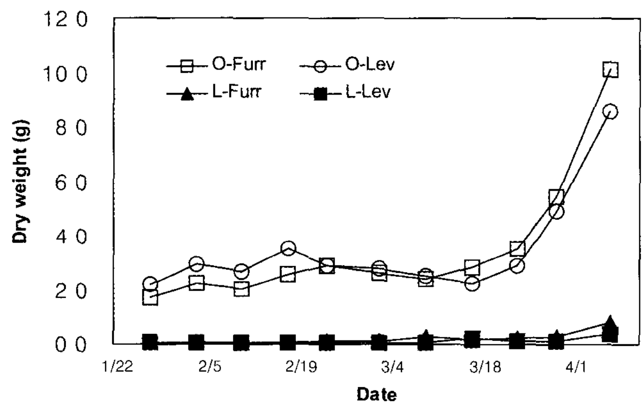

Fig. 2. Effect of the sowing practices on the dry matter accumulation of CMV after overwinter. The weight as expressed per plant O, L, Furr, and Lev represent optimal planting, late planting, furrow-sown, and levy-sown, respectively.

between furrow-sown and flat levy-sown CMV was not greatly different under optimal planting (Sep. 5), however, the dry weight of levy-sown plant was significantly reduced with delayed planting (Oct. 20). Considering the growth of underground part is related to the growth of aerial part, the more accumulated biomass of early-sown CMV should be more tolerant to freezing temperature. Besides sowing date, sowing method was also crucial to the success of overwintering ability in fall-sown CMV. There also significant difference in dry weight between the sowing method, furrow-sowing and sowing on flat bed. Dry weight of furrow-sown plants was greater than that of flat bed-sown plant after overwinter. The plants sown on furrow were sheltered from cold

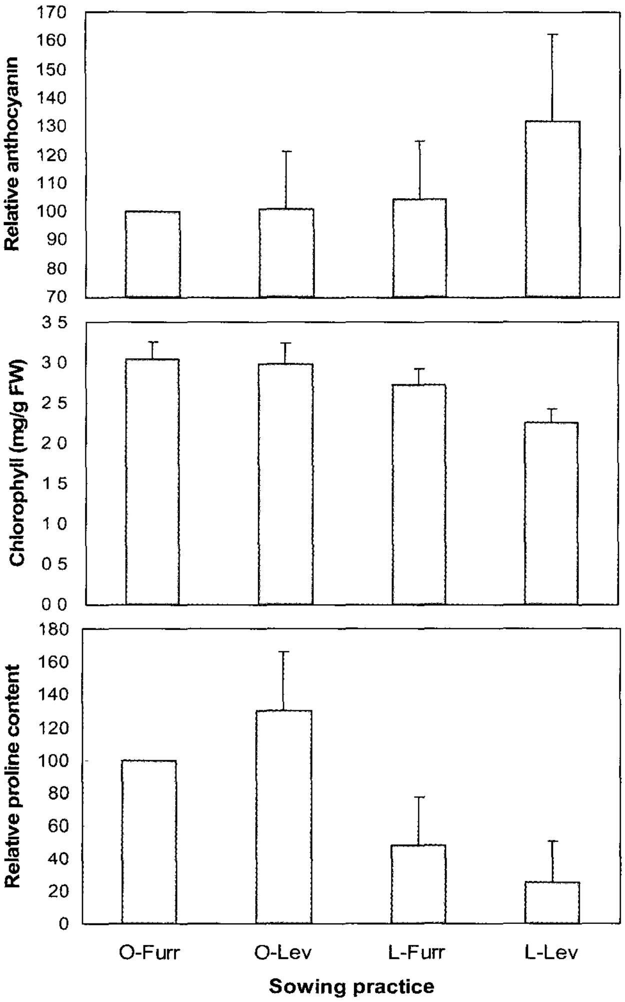

Fig. 3. Comparison of anthocyanins, chlorophyll, and proline content in CMV after overwinter under different sowing practices. Anthocyanins and proline content were expressed as a percentage of O-Furr treatment.

wind and less damaged by cold temperature and may be regrown next spring.

Anthocyanins content, an indicator for the cold stress in plant (Christie *et al.*, 1994), was higher in the late-sown plant, especially sown on the flat-bed, as compared to the furrow-sown plant (Fig. 3). On the other hands, chlorophyll content was lower in the plant sown on flat-bed at late sowing time. The difference in the level of chlorophyll, however, was reduced after mid-March (Fig. 3). Proline content was distinctly different between optimal (recommended) and late sowing. The content was greater in the plant sown at the optimal timing (Fig. 3). Sucrose content was inversely higher in the plant sown at the late timing. The relative water content related to the sucrose level showed the highest value in plant sown on flat bed at the late timing. The results suggested that CMV plant sown under unfavorable condition decreased proline although sucrose content was increased (Table. 3).

Critical freezing temperature which directly affects the survival of CMV was different according to the collection (genotype) and growth stage. However, the degree of difference among genotypes was less than that of sowing practice (sowing date and sowing method). The evaluation of LT_{50} based on the amount of electrolyte leaching reflected that CMV leaves at different stage have different tolerance to the freezing temperature. In general, older leaves from lower position exhibit weak resistance to low temperature. This result implies that the metabolic activities which may contribute the low temperature resistance were lower in old leaves than upper leaves which emerged later. The variation in LT_{50}, however, was not in accordance the latitude of collected sites.

In conclusion, the differences in tolerance to freezing temperature were not conspicuous among CMV genotypes, however, the differences between genotype (collections at different regions) were due to the plant architecture, mainly to the leaf angle. The crouching genotype collected at central region of Korean peninsula, which showed excellent freezing tolerant, has planophile leaves. The feature of internal constituents of CMV genotypes did not show any noticeable differences with respect to the freezing tolerance which evaluated by leaf disc leaching experiment. To overcome the poor overwintering capacity, tolerant genotype should be developed by selection with considering the plant architecture. The reduction of CMV growth during overwintering period was ameliorated with furrow-sowing under late-sown condition, therefore, when the CMV is inevitably sown late after recommended time, the seeds should be sown on furrow to overcome the cold stress.

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