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Effect of Planting Date on Freezing Tolerance and Winter Survival of Canola (*Brassica napus L.*)

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播種時期가 케놀라의 耐凍性과 越冬率에 미치는 影響

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ABSTRACT : Planting date is the most important factor in determining winter survival of crop plants. The objective of this study was to explore the effect of planting date on the development of freezing tolerance and winter survival of canola. Six winter cultivars were planted at three different dates during the fall on the Michigan State University Research Farm at East Lansing, MI. Freezing tolerance was determined by ion leakage tests every 15 days after planting until middle of November. Winter survival was evaluated by counting the live plants in the fall and next spring. Planting date as well as cultivar treatment had a significant effect on freezing tolerance and winter survival A different pattern in development of freezing tolerance was observed for different planting dates. There was a high correlation between freezing tolerance and winter survival suggesting that freezing tolerance could be a useful predictor for winter survival

Key Words : Freezing tolerance, Winter hardiness, Canola, Planting date

The name canola was coined by Canadians in 1979 to apply to rapeseed cultivars with low erucic acid and low glucosinolate. Such cultivars may be further described as 'double low' or 'double zero' rapeseed. Consequently, the term canola can be used to describe any rapeseed cultivars with low erucic acid content in the oil and no more than 30 micromoles per gram of glucosinolate in the defatted meal[17]. Canola production is affected by cold temperature in various ways. Winter hardiness of canola is of great concern to agronomists in cold northern temperate regions of the world because winter injury severely limits their production area.

Of all the factors related to winter hardiness, direct freezing injury has been cited as the principal cause of winter killing[14], and the process of cellular dehydration leading the destruction of the membrane during the freezing-thawing cycle is the most disruptive and injurious component of freezing injury. Cold acclimation, exposure of plant to low but nonfreezing temperature, dramatically increase freezing tolerance [1,9,16]. Cold acclimation may be influenced by

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radiation, temperature, photoperiod, precipitation, and developmental stage of plants, with different optimum conditions for different species and cultivars[19]. Of all the environmental factors influencing cold acclimation, temperature is most important[11,12]. Low, nonfreezing temperatures are conducive to an increase in hardiness in the fall, and warm temperatures are responsible for its decrease in the spring. Generally, it is thought that most plants will acclimate as temperatures are gradually lowered below 10°C. However, during acclimation the progressive decline in temperature from relatively high temperatures in early fall, followed by low, nonfreezing temperature in late fall and early winter, followed by freezing temperature in winter is extremely important in the acclimation process. The development of freezing tolerance in plants during the fall(acclimation) and its loss during spring (deacclimation) roughly follows a cyclical pattern[21]. In studies by Ruelke and Smith[15] plants began to develop freezing tolerance in early to mid-September as measured by electrical conductance. The development of tolerance continued through autumn until late November, and reached a maximum shortly after permanent freezing of the soil surface after which weekly air temperature remained below freezing. A high level of freezing tolerance was maintained from early December to mid-February, when snow cover provided protection from freezing. Tolerance began to decrease in mid-February with the onset of warmer temperatures and reduced snow cover. Thereafter it began to drop rapidly after the snow had disappeared and the soil surface thawed in late March[15,20]. Winter kill usually occurs during late winter and early spring, when the snow cover has disappeared and plants are exposed to extreme temperature fluctuations above and below freezing. By this time the plants have lost some freezing tolerance in response to warmer temperature and may be unable to reharden satisfactorily, or temperature drop is so rapid that they do not have time to reharden[18].

Although there is considerable information available on the effect of planting date on the performance of winter rapeseed[2,3,4,5,6,7,8,10], most are concerned with agronomic factors such as yield, crop quality, and winter survival. Limited information is available on the mechanism of cold hardiness and increase in freezing tolerance as affected by different planting dates. Differences in planting date and exposure of plants to different environmental conditions at different stages of growth influence their capacity for winter survival. An understanding of how plants respond to different planting dates will provide us clues for improving winter hardiness by both cultural practice and breeding strategies.

The objectives of this research were to determine the effect of planting date on the development of freezing tolerance and eventually, on winter survival of canola. In addition, the correlation between the freezing tolerance and winter survival was also examined.

Materials and Methods

Six winter canola cultivars (WRG86, CDH3, Duobul, Ceres, Accord, and KWC4113) which had a representative winter survival values (0 to 72%) in the previous experiment were planted at Michigan State University Agronomy Farm in East Lansing, Michigan at three planting dates, Aug. 25, and Sept. 10, and Sept. 25 in 1993. Seed of each cultivar was planted at the rate of 5.6 kg/ha in a five-row plot 6 m long and 92 cm wide.

All of the six cultivars planted on Aug. 25 three of the six cultivars of Sept. 10 and Sept. 25 planting were used for freezing test to see the effect of planting date on freezing tolerance. Freezing tests were performed 15 (Sept. 10), 30 (Sept. 25), 45 (Oct. 10), and 75 (Nov. 13) days after planting (DAP) for the Aug. 25 planting. For the Sept. 10 planting, freezing tests were performed at 15 (Sept. 25), 30 (Oct 10), and 60

DAP (Nov. 13), and for the Sept. 25 planting, at 15 (Oct. 10) and 45 DAP (Nov. 13).

Freezing tolerance of leaves was determined by the method of Sukumaran and Weiser[17] Three different plants were collected from each plot in the field and transferred to the laboratory immediately. Samples of three excised leaves from each plants were placed in a stoppered culture tube maintained in a low temperature bath (Masterline Model 2095, Forma Scientific) at 2°C. The uppermost leaves of each sampling time were used for the consistency of the experiment. Freezing was initiated by the addition of ice chips to each tube. After a 2 hour equilibration period, the bath temperature was lowered manually in 1°C increments every 30 min. Samples were withdrawn at 1 hour intervals until the temperature reached -18°C, and placed on ice for several hours, then removed and thawed overnight in a cold room at 2°C. Freezing damage was estimated by the electrolyte leakage test. Five ml of distilled water were added to each tube and the samples were shaken gently for 3 hours. Conductivity of the resulting solution was measured using a conductance meter (YSI model 35) at room temperature. A value for 100% leakage was obtained by freezing each sample at -80°C overnight, then reextracted in the original solution. A plot of temperature versus percent electrolyte leakage was used to determine the value for 50% electrolyte leakage, which was defined as LT_{50} Winter survivals were estimated by counting the number of live plants in each plot (3 row × 1m long each) in the fall(Oct. 15, 1993) and again in the spring of the next year (Apr. 15, 1994). Percent survival was recorded as (no. of plants per plot in spring/no. of plants per plot in fall) × 100.

Results and Discussion

Weather Conditions : The winter of 1993-1994 was colder than that of 1992-1993, with a minimum air temperature of -29°C (Jan. 19, 1994), compared with a low of only -22°C for the winter 1992-1993. The coldest 10-day mean daily minimum temperature in 1992-1993 was -16.5°C (end of Feb. 1993), compared to -22° C in 1993-1994(middle of Jan. 1994).

The overall pattern of air temperature changes in the 1993-1994 growing season was as follows: warm temperatures (above 10°C of 10-day mean daily minimum temperature) were maintained until the middle of the September. Then, the temperature decreased slowly from 10°C to freezing through the middle of September to the beginning of November. Freezing temperatures

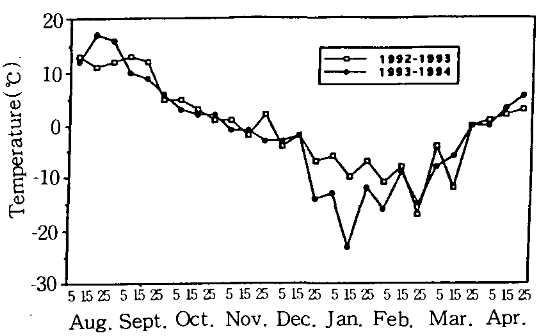

Fig. 1. Ten-day mean daily minimum temperatures at East Lansing, MI during the winter in 1992~1993 and 1993~1994.

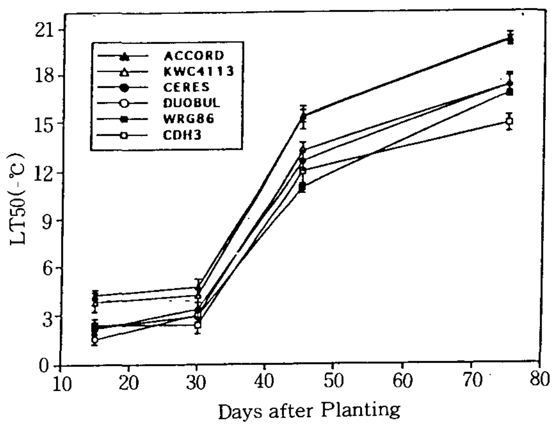

Fig. 2. Changes in freezing tolerance of six winter canola cultivars planted Aug. 25, 1993. Freezing tests were conducted at 15, 30, 45, and 75days after planting.

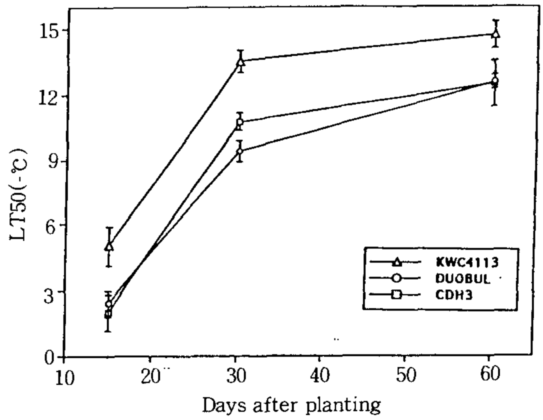

Fig. 3. Changes in freezing tolerance of three winter canola cultivars planted Sept. 10, 1993. Freezing tests were conducted at 15, 30, and 60 days after planting.

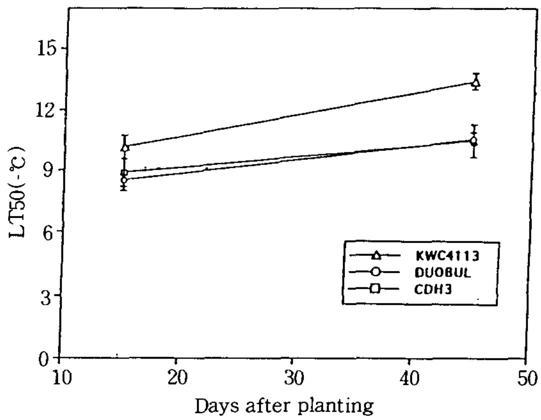

Fig. 4. Changes in freezing tolerance of three winter canola cultivars planted Sept. 25, 1993. Freezing tests were conducted at 15 and 45 days after planting.

occurred at the beginning of November, developed to around -2°C until there was a sudden temperature drop to -14.2°C in the end of December. The coldest time of the year occurred in mid-January, and these severe conditions lasted until the end of February. In March, the temperature was still maintained at freezing levels through the end of the month, then slowly increased to 0°C until the beginning of April.

Increase of Freezing Tolerance under Field Conditions : For the canola planted Aug. 25 (Fig. 2), no increase in freezing tolerance was detected until 30 DAP (Aug. 25-Sept 25). Thereafter, a rapid increase in freezing tolerance occurred during 30 to 46 DAP (Sept. 25-Oct. 13) followed by gradual increase until 75 DAP (Oct. 10-Nov. 10). There were 3 distinct groups of cultivars with different LT_{50} values. CDH3 and WRG86 belonged to the least hardy group, Duobul and Ceres belonged to medium hardy group, and Accord and KWC4113 belonged to the most hardy group. The very hardy cultivars, KWC 4113 and Accord, attained their maximum LT_{50} so values of -20°C, while that of the susceptible cultivar, CDH3 was -15°C on Nov.13.

For the Sept. 10 planting(Fig. 3), a rapid increase in freezing tolerance occurred during 15 to 30 DAP (Sept. 25-Oct 10) followed by very steady increase during 30 to 60 DAP (Oct 10-Nov. 13). However, the maximum freezing tolerances of cultivars in these plots were much less than that of the same cultivars planted Aug. 25. For example, KWC4113 planted Sept. 10 attained an LT_{50} value of -15°C whereas the same cultivar planted Aug. 25 attained -20°C. For the Sept. 25 planting (Fig 4), most of the freezing tolerance retained at the end of hardening process was gained during the first 15 DAP (Sept. 25-Oct. 10), followed by steady increase to a maximum of -13°C for the most hardy cultivar, KWC4113, and only -8.5°C for less hardy cultivars.

The effect of planting date on freezing tolerance is shown in Fig. 5. Each data point represents the overall means of six or three cultivars shown in Fig. 2, Fig. 3, and Fig. 4. A dramatic increase in freezing tolerance in all three planting dates occurred during the period of Sept. 25 - Oct. 10 during which 10-day mean daily minimum temperature dropped from 8.9°C to 2.7°C.

The main points to be drawn from the data presented are (i) earlier planting resulted in a greater freezing tolerance than later planting, (ii) there were two phase of development of

Table 1. Winter survival of six winter canola cultivars, planted Aug. 25, Sept. 10, and Sept. 25, 1993

Cultivars	Planting date		
	Aug. 25	Sept. 10	Sept. 25
	--winter survival(%)--		
ACCORD	100.0a†	72.0a	40.0a
KWC4113	100.0a	70.0a	20.0a
CERES	84.0b	59.0b	6.0b
DUOBUL	93.0b	65.0b	0.0c
WRG86	79.0bc	57.0b	0.0c
CDH3	66.0c	41.0C	0.0c

† Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

Table 2. Simple correlation coefficients between freezing tolerance and winter survival of canola cultivars planted Aug. 25, Sept. 10, and Sept. 25, 1993

Planting date	Sampling date	r
Aug. 25	Sept. 10	0.54ns
	Sept. 25	0.89*
	Oct. 10	0.85*
	Nov. 15	0.94**
Sept. 10	Sept. 25	0.88*
	Oct. 10	0.96**
	Nov. 15	0.83*
Sept. 25	Oct. 10	0.87*
	Nov. 15	0.93**

† (*) and (**) simple correlation coefficient significant at 0.05 and 0.01 probability levels, respectively.
(ns) not significant at the 0.05 probability level.

freezing tolerance regardless of planting dates, an initial and rapid increasing phase followed by a steadily increasing phase, (iii) the patterns of second phase was different among planting dates, resulting a less development of freezing tolerance in later plantings, and (iv) the initiation of first phase was coincidental with that of the dropping of air temperature to around 5°C which is known to be optimal temperature for canola acclimation.

The reason that the planting date affected the development of freezing tolerance may be attributed to genetic and environmental factors, or the interaction between the two factors Also, this may be explained by difference in growth; earlier planting grew more and accumulated more photosynthetic reserve which was attributed to increase in freezing tolerance. This suggestion is supported by published reports that reduction of carbohydrate reserves reduced cold hardiness in overwintering plants[9]. Thus less accumulation of photosynthetic reserve in the later planting may inhibit further increase of freezing tolerance in second phase of freezing tolerance development in this experiment. Another possibility is that the gradual exposure to cold temperature more effective than the sudden exposure to hardening temperature. This is supported by a report by Pomeroy et al.[3] that high levels of hardiness in wheat could be rapidly induced in 4-6 days if the hardening temperature was preceded by warm temperature. The coincidence of initiation of development of freezing tolerance and dropping of the air temperature to hardening temperature supported the idea that the temperature had an important role in cold acclimation of plants. Involvement of other environmental factor than temperature to cold acclimation might be identified in further experiment.

Winter Survival and Correlation between Freezing Tolerance and Winter Survival : Although both cultivar and planting date significantly affected winter survival, the effect of planting date was greater than that of cultivar (Table 1). Earlier planting (Aug. 25) resulted in above 80% survival except for the least hardy cultivar (66%), while later planting (Sept. 25) resulted in no survival for four of the six cultivars. The hardy cultivars, Accord and KWC4113, showed 40 and 20% survival, respectively, even when planted Sept. 25, while other cultivars showed no survival. A significant correlation was observed between freezing tolerance and winter survival of cultivars in these studies (Table 2) except for the Sept. 10 sampling date from the Aug. 25 planting. Correlation coefficients ranged from

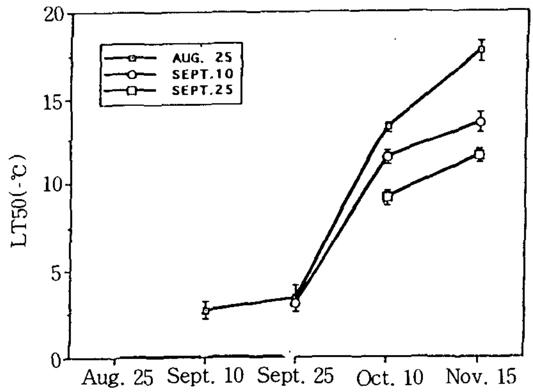

Fig. 5. Changes in freezing tolerance of winter canola affected by different planting dates. Data are overall means of six cultivars planted on Aug 25, and overall means of three cultivars planted Sept. 10 and Sept. 25. Freezing tests were performed at every 15-day or 30-day intervael, depending on planting date.

0.83* to 0.96**.

These result suggests that the freezing tolerance plays a major role in winter survival of plant and the increase of freezing tolerance by genetic or cultivation practice would promote the winter survival of crop plant to extend the marginal area of the crop cultivation, otherwise limited by the freezing temperature. In this sense, one target strategy to improve winter survival by only increasing freezing tolerance can be employed to the breeding efforts if there are difficulties to improve overall winter survival[20], In the point of cultural practice, plant growth itself may affect the winter survival of canola. Earlier planting promotes greater fall foliar growth, which covers the growing point, thus protecting it. Later planting does not provide adequate foliar coverage; thus, the growing point remains vulnerable to freezing damage. This is consistent with data that snow cover increases winter survival of canola under severe winter conditions.

The correlation between freezing tolerance and winter survival suggested that this test can be a predictors for the winter survival. Field survival is the usual method for evaluating winter survival, but results are often inconclusive due to either complete death or complete survival of all genotypes depending on the particular winter season[21]. Therefore, it would be useful to have an indirect positive test with which to screen winter hardiness. The freezing tolerance was a good indicator for the winter survival of canola and this test can be used for the screening of winter hardiness without as much difficulties as in field test.

摘 要

播種時期는 작물의 越冬律에 가장 중요한 영향을 미치는데, 본 실험은 케놀라의 파종시기에 따른 耐凍性의 증가형태 및 이러한 耐凍性이 궁극적으로 越冬律에 미치는 영향을 구명하기 위하여 수행되었다. 여섯 가지의 케놀라 品種을 8월 25일, 9월 10일과 9월 25일의 세 播種時期로 구분하여 圃場에 파종하였으며, 파종 후 15일 간격으로 11월 중순까지 잎 표본을 채취하여 실험실에서 electroleakage test 법에 의하여 耐凍性을 측정하였으며, 越冬律은 포장상태에서 가을과 봄에 걸쳐 살아있는 個體數를 세어서 算定하였는 바 그 결과는 다음과 같았다.

1. 내동성이 증가하는 형태는 播種時期에 따른 양태를 보였는데, 일찍 파종할수록 생육기 전반에 있어서 耐凍性의 증가가 일어나지 않는 반면 일찍 파종한 구에서는 꾸준한 증가세를 유지하였는데 이는 식물체의 생장정도에 따라 低溫에 반응하여 耐凍性을 증가시키는 능력에 차이가 있는 것에 기인된다고 생각된다.
2. 세 파종시기에 있어서 공히 耐凍性의 급격한 증가를 보이는 기간은 기온이 케놀라의 低溫適應에 알맞은 2~5일 때임을 미루어 볼 때 식물체의 低溫適應에 영향을 미치는 여러 가지 환경요인 중 低溫의 중요성을 시사한다고 하겠다.
3. 耐凍性과 越冬律은 긴밀한 相關關係를 보였으며, 이는 耐凍性이 越冬律을 결정하는데 있어서 가장 중요한 要因의 하나이며 이러한 실내에서의 耐凍性測定이 越冬律 向上을 위한 育種道具로 쓰일 수 있는 가능성을 확인할 수 있었다.

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