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Soybean Ecological Response and Seed Quality According to Altitude and Seeding Dates

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ABSTRACT This experiment was carried out to examine ecological response and soybean quality as affected by environmental cultivation for producing high seed quality in domestic soybean variety. The results are as follows: Under equal cumulative temperature condition, soybean plants grown in Muju showed longer days to flowering, which was an effect of the long day-length on high latitudes, and longer duration of reproductive stage as a result of low temperature within that period.

Considering apparent seed quality, 100 seed weight of soybeans grown in Muju was heavier than Miryang. Ratio of seed crack and disease-damaged seeds was lower in Muju, and these parameters decreases as planting was delayed. The protein contents did not show significant difference in terms of altitude and planting date, however, crude oil contents were higher in Miryang. An opposite trend was observed in C18:1 and C18:3. In the fatty acid composition, the proportion of C18:1 decreased as seeding date was delayed, and was higher in Miryang. Opposite observations were obtained from C18:3. The anthocyanin contents were highest on June 10 planting and higher in Muju than in Miryang. Isoflavone content was higher as seeding date was delayed and is similar accross seeding dates in Muju.

As a summary, for high seed quality production the optimum planting date was June 10, and Muju was more suitable region than Miryang.

Keywords : soybean, seeding date, region, seed quality, seed component

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Soybean (*Glycine max* (L.) Merr) originates from northeast Asia around Manchuria. Its ecotypes are well differentiated and considered to be a broadly adapted crop. Soybean is cultivated from the north latitude (52°) of China and (46°) Canada to the south latitude (36°) of Latin America. Thus, cultivation environment is closely related to plant development and has great influence on seed composition.

During the early times, soybean was classified as a short-day plant (Garner & Allard, 1920), and due to its photoperiodic response, flowering period and maturity period in most of variety are said to be delayed as latitude turns higher (Cartter & Hartwig, 1963). But results of many researches had cited that each soybean variety represents a different photoperiodic response (Johnson *et al.*, 1960; Byth, 1968). Researchers took notice of the influence of daylength influence on post flowering development, such as on flowering duration, rate of flower production and reproductive stage duration (Boote, 1977; Criswell & Hume, 1972; Fisher, 1963; Fukei & Yarimizu, 1951; Johnson *et al.*, 1960; Nagata, 1960; Patterson *et al.*, 1977; Thomas & Raper, 1976; Van Schaik & Probst, 1958).

Photoperiodic treatment by artificial and natural light showed significant shortening of growth period. Soybean grown under short daylength took fewer days from emergence to maturity, from emergence to flowering, from flowering to pod setting and flowering to maturity. However, only artificial light showed decreased number of the days from pod setting to maturity.

Consequently, effect of photoperiod treatment was only evident mainly from emergence to flowering (Johnson *et al.*, 1960). Fukui and Yarimizu (1951) reported that short-day condition induced leaf discolorisation and defoliation, and shortened maturity days which, then lead to shortenening of the period of flowering to maturity. This phenomenon was clear on soybean varieties having long maturity. In the field, long-day condition prolonged the period of flowering to pod setting (Johnson *et al.*, 1960) and flowering to flowering termination (Lawn & Byth, 1973). These results agreed with Nagata's report (1958) that photoperiodic response of soybean presented differentiation at growth stages. Also, seed weight and shape were affected by short-day treatment, which are manifested on seed weight, length, width and thickness (Fukui & Yarimizu, 1951).

For the interaction effects of temperature and daylength, Board & Hall (1984) reported that reduction of required days to first flowering was longer at high temperature (27℃) than in low (21℃). Maximum effect of temperature was larger on short-day length than long-day length and effect of temperature was larger in MG VI and MG VII than MG V. Night temperature could influence photoperiodic response which control soybean flowering (Parker & Borthwick, 1943; van Schaik & Probst, 1958). Low temperature delayed the maturity of all treated varieties across all day-length treatments and critical day length primarily because flowering was changed due to fluctuation in temperature (Steinberg & Garner, 1936). Temperature and day length affecting flowering on field was influenced by low temperature in early growth stage and day length in the later stage. Short day condition was more effective in shortening days to maturity than low temperature occurrence during fall. There was a close relationship between vegetative growth period and mean temperature. Temperature condition minimizing vegetative growth period corresponded to mean temperature on midsummer which pass the highest temperature and extension of flowering period related to low temperature on late spring or early fall (Garner & Allard, 1930). Although, growth before pod setting was closely related to degree day, days from pod setting to maturity is more important than degree day (Howell, 1960).

Early maturing types of soybean are less sensitive to day length than late maturing types (Criswell & Hume, 1972). But, Polson (1972) took notice of the delayed on maturity of early maturing type of soybean that was known to be insensitive to day length when grown under long day condition. Yoshida (1952) and Pohjakallio & Antila (1957) reported that early maturing types of soybean were insensitive to day length. The clearest difference among soybean varieties differing on days to maturity was observed on the flowering sensitivity as affected by day length, late maturing varieties are more sensitive than early maturing varieties (Major *et al.*, 1975b) Vegetative growth period, reproductive growth period and growing season are affected by seeding date and variety, and in the case of delayed seeding date, yield was evidently affected by the shortening of the reproductive growth duration, but not clearly affected by the duration of vegetative growth (Boquet *et al.*, 1983).

Apparent seed quality are affected by disease, insects and weathering after maturity (Abel, 1961). Early seeded soybeans have lower seed quality than late seeded soybean suggesting that damage duration is prolonged by the extension of seed development. Short growing period on July seeding induced low seed quality because of insect damage and presence of immature seeds (Boquet *et al.*, 1983). Feaster (1949) and Green *et al.* (1965) also reported that seed quality is affected by seeding date.

Several researchers (Fukui & Yarimizu, 1952; Johnson *et al.*, 1960; Nagata, 1958) reported that shortening of day length after flowering resulted in shortened duration of seed setting to maturity in both indeteminate and deteminate soybean varieties. Moreso, reproductive growth is affected by temperature whereas sink strength during seed filling is primarily influenced by successive short days, but its effect is slightly reduced on cooler temperatures (Fukei, 1952; Major *et al.*, 1975a, 1975b).

Although environmental condition during reproductive growth period of soybean largely influenced seed quality (Wilson, 2004), the estimated volume of accumulated components was relative to the transcription intensity of complementary gene during seed development (Wilson *et al.*, 2001a). The change on soybean seed composition are believed to be a product from the response of gene or gene product affecting character expression on environmental condition. Temperature might probably be the primary factor affecting seed quality especially during the reproductive growth period. These claims are evident when the reverse action of genotype and environment was observed on the component content of soybean seed.

It is well known that environmental conditions during reproductive growth influence soybean seed quality (Wilson, 2004). Protein and oil content of soybean seed were proven to be greatly influenced by temperature during the reproductive growth period (Wolf *et al.*, 1982). An inverse relationship between protein and oil contents and growth temperature were reported by Piper & Boote (1999). Moreover, Beatty *et al.* (1982) stated that protein content of soybean seed was affected by year, seeding date×year and seeding date×variety while oil content was affected by year, seeding date and seeding date×year. In general, it is not safe to assume that all soybean varieties will respond similarly to changes on temperature at the same manner. A temperature experiment conducted by Carter *et al.* (1986) revealed that protein and lipid content of the mature seed of closely related high protein content soybean lines have differential response to changing temperatures. This phenomenon suggests that factors aside from temperature and genes also influenced production of protein and lipid on soybean seeds (Moorman, 1990). Thus, metabolic mechanism for the formation of protein and lipid among soybean lines is changed according to G×E effect. Temperature in controlled environmental condition did not affect saturated fatty acid, specifically 16:0 and 18:0. However, a negative relationships between 18:1 and 18:2 with 18:3 are observed as temperature decreases. These relationships were evident on the seed of the germplasm N78-2245, a line having a natural recessive mutation in the gene encoding for ω-6 desaturase. The proportions of 18:1 fatty acid of N78-2245 showed wide variation at different temperature levels. A mean value of 30% was observed on low temperature condition while a 50% was obtained on high temperatures. In addition, an additive effect on the low proportion of 18:3 fatty acid was observed when the recessive gene encoding ω-3 desaturase was combined with recessive gene encoding ω-6 desaturase (germplasm N85-2176) (Burton *et al.*,1989). Many theories were formulated to explain the increasing polyunsaturated fatty acid (PUFA) as growth temperature decreases. One is that solubility of oxygen in cytoplasm controlled desaturase activity which is sensitive to temperature changed (Harris & James, 1969). Another theory states that the increase of PUFA in low temperature can be attributed to the difference in the optimum temperature requirement of desaturase (Martin *et al.*, 1986) and that membrane fluidity is controled by exposing the active site of enzyme having an effect on physical position (Inbar & Shinitzky, 1974). These theories were quite interesting but there was no theory that directly explained the biological mechanism behind the temperature effect on ω-6 desaturase. The reasonable theory in the present day is that activity of ω-6 desaturase is controlled by a series of signal transduction including reversible phosphorylation of enzyme (Topfer *et al.*, 1995). To tell the truth phosphorylation site is discovered in primary structure of ω-6 desaturase in soybean seed.

It is known that total isoflavones content in soybean seed is negatively correlated with growth temperature (Tzukamoto *et al.*, 1995). Isoflavone and its glucoside content varies according to variety and cultivation region. Significant variation was observed in Daidzin, Glycitin 7-β-glucoside, Genistin and total isoflavone content. Eldridge & Kwolek (1983) suggested that annual difference on isoflavone and its glucoside is related to climatic and environmental factors.

Black pigments on black soybean seed coat are the anthocyanins that accumulated in the palisade layer of epidermis. Anthocyanin appears in the flower of plant and skin of fruit causing the red, blue and purple color. It is a chemical compound with a special hydroxyl in sugar combined with a functional group which could be an alcohol, phenol and aldehyde.

Recently, importance of soybean food increase as function of components of soybean is elucidated. In this point high quality soybean production for enlargement of cultivation area and diversification of soybean consumption rise issue.

This experiment was carried out to examine soybean's ecological response and seed quality as affected by environmental cultivation for the production of high quality domestic soybean variety.

MATERIALS AND METHODS

This experiment was carried out at Miryang (altitude: 12 m) and Muju (altitude: 600 m) from 2005 to 2006. Varieties used were Daewon and Daepung (mid-late maturity type used for fermentation), Daol (an extremely early type) and Cheongja 3 (variety best mixed with rice). Seeding was performed on April 30 to June 30 and planting density was 142,857 plants/ha (70×20 cm, 2 plants per hill) with black vinyl mulching. Fertilization was set at 30-30-34 (N-P_2O_5-K_2O) per ha using urea, magnesium phosphate and potassium chloride. The experiment was laid out split-plot design. Data obtained included major growth stage, apparent seed quality (seed weight, seed crack, purple seed and phomopsis decayed seed) and major component of seed (protein, lipid, fatty acid, isoflavone and anthocyanin).

The seeds of soybean cultivars were finely ground using a coffee grinder (HEICO, LT1-100, Japan). The protein content was determined according to the data from the near-infrared reflectance spectroscopy (NIRS). The NIR data were collected in the range of 400 to 2500 nm at 2-nm intervals using a Foss NIRSystems spectrophotometer (model 6500, NIRSystem Inc., Silver Spring, MD) and stored as the reciprocal logarithm (log 1/R) of the reflected energy. All statistics, regressions, and predictions were performed using the WINISI software (version 1.05) from Foss NIRSystem. Coefficient of determination (r^2) and standard errors of calibration (SEC) were used to evaluate how well the calibration of the sample or multiple linear regressions fits the data.

The oil content was measured by the Soxhlet method using the Buchi B-811 extracted system (Soxhlet System: BUCHI Labotechnik, B-811, AG., Switzerland). Two grams of the pulverized seed was added to 200 mL of n-hexane in an extraction thimble and boiled for 3 h at 100℃. After cooling to room temperature in a desiccator, the extracted oil was weighed. Total oil contents were determined on a dry matter basis of the seeds. Fatty acid methyl esters (FAMEs) were prepared for gas chromatographic analysis by methylation of the extracted fat using H_2O : MeOH : toluene (1 : 20 : 10, v/v). The FAMEs were extracted with 2 mL hexane and 1 μl was injected into the gas-chromatograph, in split mode (split ratio 1:50). Fatty acid analysis was carried out on an Agilent gas chromatograph (Model 7890A GC) fitted with an automatic sampler (Model 7683B Injector) and FID detector. The conditions used were the following: HP-FFAP capillary column (30 m × 0.318 mm I.D., 0.25 μm film thickness; Agilent Technologies), temperature programmed from 150℃ for 1 min, then 150 to 230℃ at 2.5℃/min, then held for 2 min. Carrier gas was nitrogen, column flow 1.0 mL/min, inlet and detector were set at 250 and 260℃, respectively. Standard FAMEs, including palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid methyl ester, were calibrated as essentially the same procedures above mentioned.

To extract isoflavone, 1.0 g of soybean sample was mixed with 20 mL of 50% aqueous methanol in a 50 mL centrifuge bottle (Nalge Company, Rochester, NY, USA). The sample bottles were vortexed for 1 min and then shaken with shaker (Eyela, Japan) for 12 h at room temperature. The extracts were then centrifuged for 10 min at 3000 rpm using a VS-6000 centrifuge (Vision, Korea). The supernatant was filtered with a 0.45 μm membrane filter prior to HPLC. HPLC analyses were conducted on an Agilent 1100 series HPLC with diode array detection (DAD). A sample (20 μl) of the 50% methanolic extract was injected onto an analytical reverse phase C-18 column (125 mm × 4 mm, LichroCART, 5 μm, Merck KGaA). The mobile phase was composed of 0.1% acetic acid (TFA) in water (A) and 0.1% acetic acid in acetonitrile (B). The gradient conditions were as follows: 0 min, 10% B; 20 min, 20% B; 30 min, 25% B; 40 min, 35% B; 50 min, 40% B and then the column was equilibrated 10 min at 10% B between runs. Other HPLC conditions were as follow: a flow rate of 1.0 mL/min; column temperature, 30℃; detection, 260 nm; and sample size, 20 μl. Isoflavone standards calibrations were performed using an Agilent 1100 series HPLC following similar procedure previously mentioned. The twelve isoflavone standards stock solutions were prepared by dissolving in 50% methanol to give 1.0 mg/mL concentration. Calibration curves were made for each standard with ten different concentrations (0.625, 1.25, 2.5, 5, 10, 20, 40, 60, 80, 100 μg/mL). A high linearity (r^2 > 0.998) was obtained for each standards curves (Table 1).

The anthocyanin content was measured by HPLC analysis. Hand-peeled seed coats (0.2 g) of black soybean cultivars, were extracted with 20 mL of 40% methanol (1% HCl) for 2 days at 4℃, in darkness. The anthocyanin extracts were filtered through a 0.45 μm filter unit prior to HPLC analysis. HPLC analysis was conducted on an Agilent 1100 series HPLC with diode array detection (DAD). A sample (20 μl)

Table 1. Calibration curves of the 12 individual isoflavone standards

Standards	Equation	
Daidzein	y = $122.2x + 18.39$	r^2 = 0.999***
Genistein	y = $183.72x + 10.55$	r^2 = 0.999***
Glycitein	y = $94.45x - 5.51$	r^2 = 0.999***
Daidzin	y = $83.21x - 134.32$	r^2 = 0.998***
Genistin	y = $96.78x + 54.32$	r^2 = 0.999***
Glycitin	y = $32.1x - 8.69$	r^2 = 0.997***
Malonyl-Daidzin	y = $69.33x + 25.58$	r^2 = 0.999***
Malonyl-Genistin	y = $81.06x - 56.3$	r^2 = 0.999***
Malonyl-Glycitin	y = $74.15x - 35.9$	r^2 = 0.999***
Acetyl-Daidzin	y = $50.22x + 72.28$	r^2 = 0.998***
Acetyl-Genistin	y = $83.51x + 10.05$	r^2 = 0.999***
Acetyl-Glycitin	y = $59.16x + 53.39$	r^2 = 0.999***

of the crude acidic methanolic extract was injected onto an analytical reverse phase C-18 column (125 mm × 4 mm, LichroCART, 5 μm, Merck KGaA). The mobile phase was composed of 0.1% TFA in water (A) and 0.1% TFA in methanol (B). The gradient conditions were as follows: 0 min, 15% B; 2 min, 20% B; 30 min, 35% B and then the column was equilibrated 10 min at 15% B between runs. Other HPLC conditions were as follow: a flow rate of 0.8 mL/min; column temperature, 30℃; detection, 530 nm; and sample size, 20 μl.

RESULTS AND DISCUSSION

Results of soil analysis prior to the conduct of experiment is shown in Table 2. Data showed that Muju soil was more fertile than Miryang as indicated by its close to neutral pH, higher EC, organic matter, available phosphate and exchangeable cations.

Table 3 shows the mean and range of temperatures with respect to seeding date and growth stage of each variety in Muju and Miryang from 2005 to 2006. Mean temperature during the vegetative growth per seeding date was higher in Muju, showing a range difference of 1.7~2.3℃. A wider ranged on mean temperatures was observed at delayed seeding date. Temperature during reproductive stage varies among variety, however, it should be noted that mean temperature becomes lower as seeding date was delayed. Reproductive stage temperature is likewise higher in Miryang than in Muju, 2.9~3.4℃ difference was obtained. Temperature range is also wider as seeding date was delayed.

Table 4 shows the mean and daily range of temperatures during the vegetative and reproductive growth period in 2006. Trend on temperature is similar with those observed on average of 2005 and 2006.

Table 5 shows the flowering date of each variety according to seeding date in Muju and Miryang. Days to flowering across varieties were longer in Muju than in Milyang whereas shortening ratio of flowering according to seeding date was similarly higher in Muju than Miryang. This finding was brought about by the low vegetative growth temperature in Muju, as shown in Table 3. This result agrees with the report of Garner & Allard (1930) that shortening

Table 2. Result of soil chemical analysis at Miryang and Muju before experiment

Region	pH (1:5)	EC (ds/m)	O.M. (%)	Av. P_2O_5 (mg/kg)	Ex.cations ($cmol^+/kg$)		
					K	Ca	Mg
Miryang	6.1	0.81	2.65	244	0.53	5.68	1.63
Muju	6.6	1.12	3.61	576	0.50	6.48	2.37

Table 3. Mean temperature separated by growth stage according to different seeding date at Miryang and Muju for 2 years (2005~2006)

Seeding date	Variety	Vegetative stage		Reproductive stage			
		Mean temp. (℃)		Mean temp. (℃)		Daily range	
		Miryang	Muju	Miryang	Muju	Miryang	Muju
April 30	Daewon	20.7	18.9	24.2	21.5	10.4	10.0
	Daepung	20.4	18.7	24.5	21.6	10.2	9.9
	Daol	20.1	18.4	25.9	22.9	10.0	8.8
	Cheongja 3	21.0	19.2	24.0	21.1	10.7	10.3
	Mean	20.5	18.8	24.7	21.8	10.3	9.8
May 20	Daewon	22.4	20.4	23.9	20.8	11.0	10.6
	Daepung	22.3	20.3	24.3	21.0	10.7	10.4
	Daol	22.1	20.1	26.0	23.4	9.9	9.8
	Cheongja 3	22.6	20.6	23.6	20.7	11.3	10.7
	Mean	22.3	20.4	24.5	21.5	10.7	10.4
June 10	Daewon	24.1	21.8	23.3	20.5	11.5	10.9
	Daepung	24.1	21.7	23.5	20.6	11.3	10.9
	Daol	24.0	21.6	26.1	22.5	10.8	10.1
	Cheongja 3	24.2	22.2	23.0	20.1	11.7	11.0
	Mean	24.1	21.8	24.0	20.9	11.3	10.7
June 30	Daewon	25.4	23.2	22.3	19.2	12.0	11.1
	Daepung	25.5	23.2	22.3	19.5	11.8	11.1
	Daol	25.2	23.2	25.2	20.4	10.8	10.7
	Cheongja 3	25.6	23.3	22.0	18.9	11.9	11.1
	Mean	25.4	23.2	22.9	19.5	11.6	11.0

Table 4. Mean temperature seperated by growth stage according to different seeding date at Miryang and Muju in 2006

Seeding date	Variety	Vegetative stage		Reproductive stage			
		Mean temp. (℃)		Mean temp. (℃)		Daily range	
		Miryang	Muju	Miryang	Muju	Miryang	Muju
April 30	Daewon	20.4	18.8	23.9	21.4	10.7	9.5
	Daepung	20.1	18.6	24.2	20.8	10.6	9.6
	Daol	19.9	18.4	25.7	22.3	9.9	7.9
	Cheongja 3	20.7	19.3	23.8	20.6	11.1	10.0
	Mean	20.3	18.8	24.4	21.3	10.6	9.3
May 20	Daewon	22.0	20.4	23.6	20.5	11.6	10.2
	Daepung	21.8	20.3	24.0	20.6	11.1	10.0
	Daol	21.5	19.9	26.0	23.0	10.1	9.2
	Cheongja 3	22.3	20.4	23.5	20.5	11.7	10.6
	Mean	21.9	20.3	24.3	21.1	11.1	10.0
June 10	Daewon	23.3	21.2	23.4	20.1	12.4	11.0
	Daepung	23.3	21.2	23.9	20.2	11.9	10.9
	Daol	23.5	21.2	26.5	21.9	11.3	9.6
	Cheongja 3	23.4	21.5	23.3	19.9	12.5	10.9
	Mean	23.4	21.3	24.3	20.5	12.0	10.6
June 30	Daewon	25.0	22.7	22.4	19.1	12.9	11.0
	Daepung	25.3	22.7	22.3	19.1	12.6	11.0
	Daol	24.8	22.6	25.7	19.6	11.9	10.5
	Cheongja 3	25.4	22.9	21.8	18.7	12.8	10.9
	Mean	25.1	22.7	23.1	19.1	12.6	10.8

Table 5. Comparison of the two-year (2005~2006) mean days to flowering date as affected by seeding dates at Miryang and Muju.

Seeding date	Daewon		Daepung		Daol		Cheongja3	
	Miryang	Muju	Miryang	Muju	Miryang	Muju	Miryang	Muju
April 30	Jun.28	Jul.7	Jun.25	Jul.1	Jun.20	Jun.28	Jul.3	Jul.14
May 20	Jul.11	Jul.17	Jul.5	Jul.14	Jun.29	Jul.10	Jul.14	Jul.23
June 10	Jul.22	Jul.25	Jul.22	Jul.24	Jul.19	Jul.22	Jul.26	Aug.2
June 30	Aug.4	Aug.7	Aug.5	Aug.6	Aug.2	Aug.7	Aug.7	Aug.11
Flowering days	49	53	49	51	42	48	53	58
Shortening days	23	29	19	24	18	20	26	26
Shortening ratio (%)	38	48	32	40	29	33	43	44

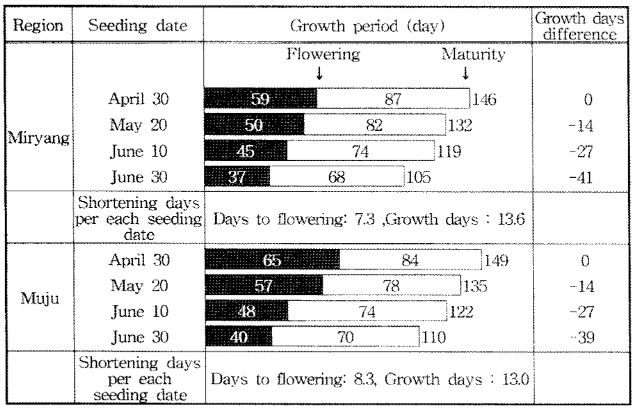

Fig. 1. Comparision of the shortening day of growth period according to seeding dates at Miryang and Muju during two years (2005~2006).

of vegetative growth period is influenced by temperature condition. Temperature during vegetative growth should reach the mean temperature of mid-summer to have normal flowering days. An extension of flowering period can be expected if temperature is similar to the temperatures of late spring and early fall.

Figure 1 presents the growth days according to seeding date in Muju and Miryang. Seeding conducted on both Muju and Miryang at April 30, May 20 and June 10 seeding showed same growth day difference. But June 30 seeding showed wider difference in Muju.

Growth day was shorter in Miryang than Muju, 13.6 and 30 days, respectively. The prolonged growth day in Muju was the result of the slightly extended days to flowering in the late seeding which was probably caused by daylength since temperature is optimum. Generally, long growth period is a factor for increasing yield. Boquet *et al.* (1983) reported that yield of late-seeded soybeans were dependent on factors affecting the reproductive stage and not that of the vegetative stage. However, it must be noted that vegetative, repro-

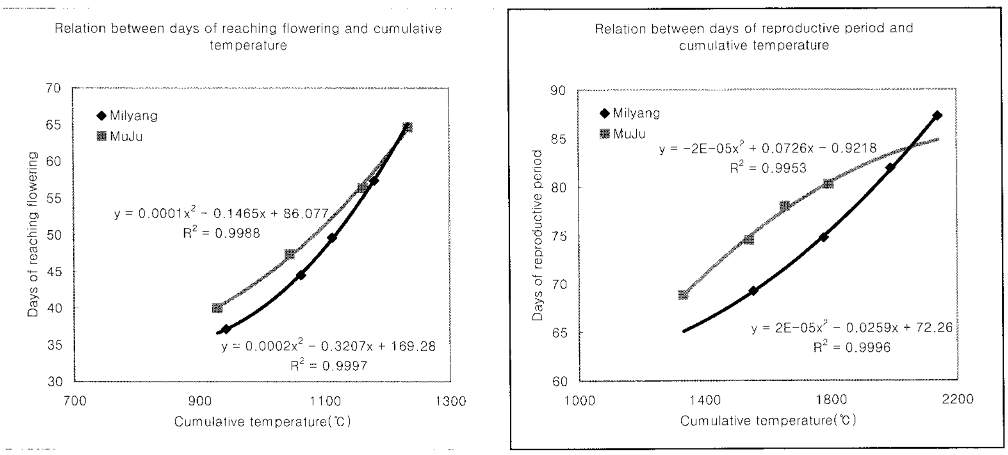

Fig. 2. Relationship between cumulative temperature and days to reach two growth stages at Miryang and Muju in two years.

Table 6. Mean temperature and rainfall during each reproductive stage according to different region and seeding date in 2006

Region	Seeding date	Variety	Mean temperature (℃)			Rainfall (mm)		
			R2~R4	R4~R6	R6~R8	R2~R4	R4~R6	R6~R8
Miryang	April 30	Daewon	24.3	28.1	20.5	551.0	118.0	79.5
		Daepung	23.9	28.2	21.6	573.0	90.5	107.0
		Daol	23.4	24.4	28.6	300.5	278.5	90.5
		Cheongja 3	25.3	27.6	19.9	411.5	118.0	79.5
	May 20	Daewon	26.4	26.8	19.2	268.5	127.0	70.5
		Daepung	25.5	27.7	19.9	297.0	118.0	79.5
		Daol	24.1	26.2	27.3	460.0	91.5	121.0
		Cheongja 3	26.4	26.3	19.1	110.0	127.0	70.5
	June 10	Daewon	28.1	24.3	18.9	33.5	107.5	69.0
		Daepung	27.9	26.2	19.4	12.5	127.0	70.5
		Daol	25.8	29.3	25.3	91.5	21.5	106.0
		Cheongja 3	28.5	23.8	18.9	70.0	99.5	69.0
	June 30	Daewon	27.8	21.5	18.5	118.0	79.5	486.1
		Daepung	27.6	21.3	18.7	118.0	79.5	0.0
		Daol	29.4	26.8	22.3	19.0	117.5	36.5
		Cheongja 3	27.2	20.7	18.3	118.0	79.5	19.0
Muju	April 30	Daewon	22.6	24.7	18.1	482.5	128.5	127.5
		Daepung	22.2	24.4	17.1	532.5	165.5	43.0
		Daol	22.1	22.9	24.0	415.0	167.5	158.5
		Cheongja 3	23.5	23.3	16.5	243.0	152.0	37.0
	May 20	Daewon	23.8	22.3	16.5	169.5	160.0	29.0
		Daepung	23.3	23.1	16.4	292.5	155.5	37.0
		Daol	21.9	25.4	22.3	438.5	21.0	160.0
		Cheongja 3	25.1	20.9	16.4	97.0	155.5	28.5
	June 10	Daewon	24.9	19.6	16.7	52.0	136.0	26.0
		Daepung	24.9	19.8	18.7	45.5	142.0	28.5
		Daol	25.2	24.0	18.0	21.5	146.0	43.0
		Cheongja 3	24.7	19.0	18.6	128.5	102.5	26.0
	June 30	Daewon	23.6	17.2	16.9	152.0	37.0	0.0
		Daepung	23.6	17.1	16.9	152.0	37.0	0.0
		Daol	24.2	18.8	16.5	146.0	45.5	28.5
		Cheongja 3	23.1	16.4	17.0	152.0	37.0	0.0

ductive growth and entire growth period are affected by seeding date and variety.

In soybean, factors promoting flowering are temperature and daylength. Higher temperature and shorter daylength promote flowering. Therefore, flowering in Muju field situated on altitude 600 m, a higher latitude than Miryang, are delayed due to lower temperature and longer daylength. Days to flowering, ripening period and cumulative temperature according to seeding date in Muju and Milyang are shown in Figure 2. Here, days required to reach flowering on the same cumulative temperature at each seeding date was longer in Muju as seeding date was delayed.

The longer days to reach flowering in Muju was due to the effect of longer daylength (photosensitivity) because in applying to a general idea of cumulative temperature, temperature factor in promoting flowering are removed. Similarly, days from flowering to maturity was also longer in Muju, because of the longer ripening period presenting in lower temperature while high ripening temperature results short ripening period by fast genesis and moving of reservoir and early exhaustion of enzyme activity.

Table 6 showed the mean temperature and rainfall at each ripening stage after flowering in Muju and Miryang.

Table 7 and Table 8 showed apparent seed quality accord-

Table 7. Apparent quality of seeds as affected by seeding dates and cultivated regions

Seeding date	Variety	100 seed weight (g)		Crack seed (%)		Purple seed (%)		Phomopsis (%)	
		Miryang	Muju	Miryang	Muju	Miryang	Muju	Miryang	Muju
April 30	Daewon	24.6	28.5	3.6	2.5	0.5	0.2	0.9	0.4
	Daepung	21.2	21.7	31.2	4.9	3.5	2.8	0.4	1.6
	Daol	34.6	36.5	38.1	21.6	1.6	6.0	13.6	12.4
	Cheongja 3	35.2	36.0	78.4	15.6	-	-	1.8	0.9
	Mean	28.9	30.7	37.8	11.1	1.9	3.0	4.1	3.8
May 20	Daewon	21.7	27.5	0.9	2.1	0.6	0.2	0.6	0.0
	Daepung	20.7	21.2	24.4	1.3	1.0	1.3	1.1	0.9
	Daol	32.1	36.1	8.9	10.1	0.5	1.3	8.7	5.1
	Cheongja 3	34.4	34.1	66.4	7.6	-	-	0.6	0.2
	Mean	27.2	29.7	25.1	5.3	0.7	0.9	2.7	1.5
June 10	Daewon	21.8	26.7	1.3	2.4	0.1	0.2	0.2	1.3
	Daepung	20.1	20.5	5.6	1.7	1.0	2.1	0.2	1.2
	Daol	30.5	32.9	10.3	6.4	0.0	0.3	3.5	4.5
	Cheongja 3	34.3	34.8	57.4	2.6	-	-	0.7	0.5
	Mean	26.6	28.7	18.6	3.3	0.4	0.8	1.1	1.9
June 30	Daewon	22.1	26.8	0.0	3.2	0.0	0.4	0.0	0.2
	Daepung	19.9	18.7	6.4	1.6	0.9	0.6	0.1	0.2
	Daol	28.4	33.3	11.5	3.2	0.0	0.4	0.0	4.5
	Cheongja 3	34.1	32.4	40.3	1.3	-	-	0.0	0.8
	Mean	26.1	27.8	14.5	2.3	0.3	0.5	0.0	1.4
Mean		27.2	29.2	24.0	5.5	0.8	1.3	2.0	2.1

Table 8. Significance analysis of seeding date, variety, region and year on seed quality of soybean seed

Source	F-Value			
	Seed weight	Crack	Purple	Phomopsis
Seeding date (A)	17.5**	21.95**	27.68**	13.16**
Variety (B)	521.71**	80.92**	35.89**	60.74**
Region (C)	47.17**	151.52**	8.05**	0.16
Year (D)	18.58	4.05	13.41	19.14
A×B	1.78	3.3**	10.16**	8.52**
A×C	0.52	4.33**	1.61	2.27
B×C	18.08**	65.76**	7.62**	0.15
A×B×C	0.95	1.39	4.84**	1.19

ing to seeding date and region. Seed weight across regions was lighter as seeding date was delayed. Seed weight in Muju was heavier than in Miryang. Opposite trends between seed crack and seed weight was obtained. Seed crack decreased as seeding date was delayed and have higher occurrence in Miryang than in Muju. This phenomenon come to the conclusion that reservior accumulate in expected size of seed coat when surplus reservoir in plant accumulate in reduced pods. Purple seed and phomopsis decayed seed decreased as seeding date was delayed.

Seed weight variation of surveyed soybean variety according to seeding date in Muju and Miryang is shown in Table 9 and Figure 3. There was a significant negative correlation between seed weight and mean temperature from R2 to R4 stages in all variety. Seed weight increased as mean temperature from R2 to R4 stage decreased to 22.0℃, which was greatly manifested on Daewon variety. At maturity time, Daewon variety had an average increase of 1.79 g as mean temperature decreased by 1℃. On the other hand, increases on seed weight per 1℃ decrease on temperature for Daepung, Daol and Cheongja 3 were 0.76 g, 0.94 g and 0.64 g, respectively.

To elucidate the climatic parameters affecting 100 seed weight, separate analysis were done on the three stages of seed ripening namely: R2~R4, R4~R6 and R6~maturity, utilizing the stepwise method of multiple regression (Table 10). Seed weight of Daewon obtained a significant negative

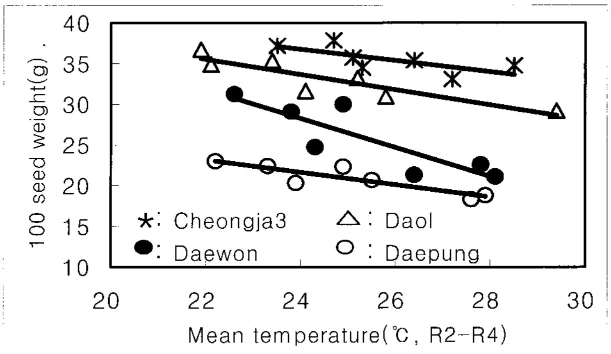

Fig. 3. Change in seed weight according to mean temperature from R2 to R4 of four soybean varieties.

Table 9. Relationship between mean temperature in the reproductive growth period and seed weight

Variety	R2~R4 stage		R4~R6 stage		R6~R8stage	
	r-value	Reduction ratio of seed weight (g/℃)	r-value	Reduction ratio of seed weight (g/℃)	r-value	Reduction ratio of seed weight (g/℃)
Daewon	0.84*	-1.79	-	-	0.65	-1.98
Daepung	0.89**	-0.76	-	-	0.53	-0.57
Daol	0.90**	-0.94	0.65	-0.82	-	-
Cheongja3	0.71	-0.69	-	-	-	-

Table 10. Analysis of meteorological factors influencing 100 seed weight of soybeans using multiple regression (stepwise)

Variety	Item	Partial R-square	Model R-square	F Value	Pr > F
Daewon	Mean temp. (R2~R4 stage)	0.738	0.738	14.07*	0.0133
	Mean temp. (R4~R6 stage)	0.243	0.981	50.05**	0.0021
	100 seed weight = 90.8 − 1.89 × Mean temp. (R2 − R4) − 0.71 × Mean Temp. (R4~R6) (R^2=0.981)				
Daepung	Mean temp. (R2~R4 stage)	0.777	0.777	17.42**	0.0087
	Rainfall (R2~R4 stage)	0.189	0.966	22.50**	0.0090
	100 seed weight = 52.8 − 1.22 × Mean temp. (R2 − R4) − 0.006 × Rainfall (R2~R4) (R^2=0.966)				
Daol	Mean temp. (R2~R4 stage)	0.813	0.813	21.78**	0.0055
	100 seed weight = 56.1 − 0.935 × Mean temp. (R2~R4) (R^2=0.813)				
Cheongja 3	Mean temp. (R2~R4 stage)	0.497	0.497	4.94	0.0769
	100 seed weight = 53.3 − 0.687 × Mean temp. (R2~R4) (R^2=0.497)				

correlation with mean temperature at R4~R6 stage and on rainfall at R4~R6 stage. Mean temperature and rainfall were able to estimate 100 seed weight of Daewon and Daepung variety with 98.1% and 96.6% probability, respectively. For the case of Daol variety, mean temperature during R2~R4 stage is enough to estimate seed weight. On the contrary, estimation of 100 seed weight using rainfall and temperature data seemed to be not applicable with Cheongja 3.

Relationship between phomopsis occurrence and rainfall is shown Figure 4 and 5. Phomopsis occurrence in Daol, an early maturing type, was closely related to rainfall during R2 stage to maturity and it increases as rainfall increases. On the other hand, phomopsis occurrence in late maturity type (Daewon, Daepung and Cheongja 3) was closely related to rainfall during the R4~R6 stage. In this period, a rainfall over 100 mm had increased the infection ratio. Although degree of seed quality is dependent on seed development period, disease, insect damage and weathering after maturity (Abel, 1961), for this experiment, it is believed that phomopsis and purple disease becomes prevalent on condition of high temperature and rainfall during the ripening period and thereafter damage of seed occurred. Also, Boquet *et al.* (1983) reported that low seed quality on early seeded soybean was due to longer damaging period which resulted from longer seed development.

Table 11 shows the protein contents of soybean seed as affected by seeding date and region. Protein contents of each variety decreases as temperature decreases but there

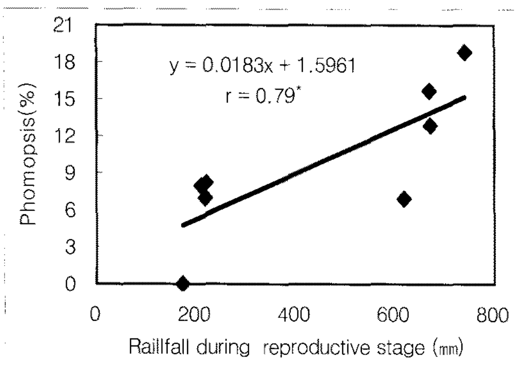

Fig. 4. Relationship between rainfall at reproductive stage and phomopsis infection ratio in Daol, the early maturing variety.

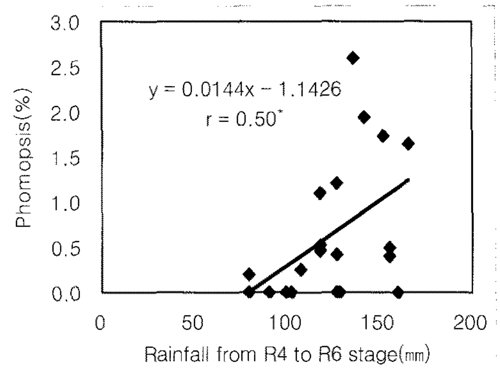

Fig. 5. Relationship between rainfall during R4~R6 stage and phomopsis infection ratio in late maturing soybean varieties Daewon, Daepung and Cheongja 3.

Table 11. Protein contents of soybean seed as affected by seeding dates and cultivated regions (Unit: %)

Region	Seeding date	Variety				Mean
		Daewon	Daepung	Daol	Cheongja3	
Miryang	April 30	42.2	40.9	45.3	44.6	43.2a
	May 20	41.3	41.0	45.2	43.1	42.7ab
	June 10	40.7	39.3	43.2	42.6	41.5ab
	June 30	39.2	40.0	42.4	42.8	41.1b
	Mean	40.9	40.3	44.0	43.3	42.1
Muju	April 30	41.1	38.6	45.5	40.9	41.6ab
	May 20	41.0	40.4	44.7	41.6	41.9ab
	June 10	42.0	41.1	43.4	44.1	42.7ab
	June 30	43.1	40.5	44.9	42.4	42.7ab
	Mean	41.8	40.2	44.6	42.2	42.2
Mean		41.3c	40.2c	44.3a	42.8b	-

was no significant difference among seeding dates and between regions. Wolf *et al.* (1982) reported that growth temperature have an influence on protein and lipid contents of soybean seed while Carter *et al.* (1986) stated that protein contents tends to become lower as growth temperature becomes higher.

Relationship between protein and lipid contents and mean temperature at R4~R6 stage in soybean suited for fermentation (Daewon and Daepung) as affected by seeding date in Muju and Miryang is shown in Figure 6. Data revealed that despite the negative relationship between protein and lipid contents with mean temperature, protein contents decreased and lipid contents increased as mean temperature from R4 to R6 stage increased. Protein content decreased by 0.44% and lipid content increased by 0.26% as mean temperature increased by 1℃. Bahman *et al.* (1977) reported that protein and lipid synthesis is minimal from 10 days to 30 days after flowering. The part of lower protein and higher lipid content in Figure 6 corresponded to the early seeding date.

Lipid contents of soybean seed as affected by seeding date and region is shown in Table 12 and Table 13. Lipid contents increased as seeding date was advanced and was higher in Miryang than in Muju. This results agreed with Carter *et al.*'s report (1986) that lipid content tend to become lower as growth temperature during maturity period becomes

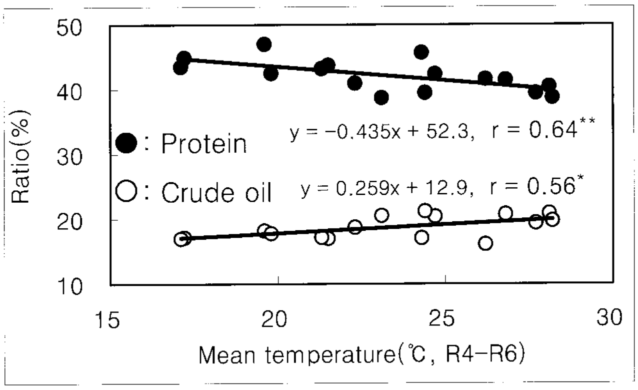

Fig. 6. Relationship between mean temperature during R4~R6 stage and protein, crude oil contents of two soybean varieties (Daewon, Daepung).

Table 12. Crude oil contents of soybean seed as affected by seeding dates and two cultivated regions (Unit: %)

Region	Seeding date	Variety				Mean
		Daewon	Daepung	Daol	Cheongja3	
Miryang	April 30	19.9	18.6	17.7	17.0	18.3
	May 20	19.8	19.1	17.1	17.1	18.2
	June 10	19.5	19.5	17.4	17.3	18.4
	June 30	19.0	18.5	15.7	17.0	17.6
	Mean	19.5	18.9	17.0	17.1	18.1
Muju	April 30	17.8	17.4	16.6	17.4	17.3
	May 20	18.3	17.2	16.2	16.8	17.1
	June 10	16.9	16.1	15.3	14.8	15.8
	June 30	15.9	16.1	13.8	14.0	14.9
	Mean	17.2	16.7	15.5	15.7	16.3

Table 13. Significance analysis of seeding date, variety and region on crude oil contents of soybean seed (Unit: %)

Source	Seeding date		Variety		Region	
F-value	5.68**		12.79**		38.49**	
Mean value	April 30	17.7a	Daewon	18.3a	Miryang	18.1a
	May 20	17.6a	Daepung	17.8a	Muju	16.2b
	June 10	17.1ab	Daol	16.4b	-	-
	June 30	16.2b	Cheongja3	16.2b	-	-

lower.

Table 14, 15, 16 and 17 shows the amount of unsaturated fatty acid as affected by seeding date and region. C18:1 contents decreased as seeding date was delayed. Oppositely, C18:3 contents increased as seeding date was delayed and was higher in Muju than Miryang. These results concur with the reports that cultivation year and region affects C18:3 fatty acid contents of soybean and that it becomes lower as temperature at maturity period becomes higher (Cherry *et al.*, 1985). Furthermore, high temperature during maturity period likewise decreased the content of C18:2 and C18:3 on soybean seed and increased C18:1 contents (Howell & Collins, 1957; Wolf *et al.*, 1982). A report by Cherry *et al.* (1984) stated that an inverse relationship between C18:1 and C18:3 existed in crude oil of soybean whereas Primomo *et al.* (2002) reported that cultivation year mainly influenced level content of all fatty acid while cultivation region influenced contents of C18:1 and C18:3. Reciprocal effect of genotype and cultivation year influenced all fatty acid while reciprocal effect of genotype, cultivation year and region only significantly influenced contents of C18:1, C18:2 and C18:3.

Table 18 showed isoflavone contents as affected by seeding date and region. Isoflavone contents was higher as

Table 14. C18:1 contents of soybean seeds as affected by seeding dates and cultivated regions (Unit: %)

Region	Seeding date	Variety				Mean
		Daewon	Daepung	Daol	Cheongja3	
Miryang	April 30	29.6	20.0	28.4	22.4	25.1
	May 20	26.5	19.3	37.9	21.1	26.2
	June 10	23.3	19.9	31.7	20.6	23.9
	June 30	19.3	19.2	23.3	20.8	20.7
	Mean	24.7	19.6	30.3	21.2	24.0
Muju	April 30	26.6	19.7	36.8	22.3	26.3
	May 20	25.1	20.6	33.6	21.3	25.2
	June 10	23.4	20.1	25.8	20.3	22.4
	June 30	21.4	20.4	23.0	19.9	21.2
	Mean	24.1	20.2	29.8	21.0	23.8
Mean	-	24.4b	19.9c	30.1a	21.1bc	-

Table 15. C18:2 contents of soybean seeds as affected by seeding dates and cultivated regions (Unit: %)

Region	Seeding date	Variety				Mean
		Daewon	Daepung	Daol	Cheongja3	
Miryang	April 30	51.5	57.9	50.7	56.7	54.2
	May 20	54.4	59.0	42.3	57.0	53.2
	June 10	56.1	58.5	48.3	56.9	55.0
	June 30	58.0	58.6	53.7	56.5	56.7
	Mean	55.0	58.5	48.8	56.8	54.8
Muju	April 30	53.1	58.0	43.2	55.5	52.4
	May 20	54.0	57.0	45.6	56.3	53.2
	June 10	54.5	56.8	51.6	56.3	54.8
	June 30	55.4	56.9	52.2	55.6	55.0
	Mean	54.2	57.2	48.1	55.9	53.9
Mean		54.6b	57.8a	48.4c	56.4ab	

seeding date was delayed in Milyang and was similar among seeding dates in Muju except with that of the June 30 seeding. These results agreed with Tsukamoto *et al.*'s report (1995) that isoflavone content becomes higher as temperature at maturity period becomes lower.

Table 19 and 20 showed anthocyanin contents as affected by seeding date and region. Anthocyanin contents was higher in Muju and was higher as temperature during maturity

Table 16. C18:3 contents of soybean seeds as affected by seeding dates and cultivated regions (Unit: %)

Region	Seeding date	Variety				Mean
		Daewon	Daepung	Daol	Cheongja3	
Miryang	April 30	6.6	9.3	7.4	8.2	7.9
	May 20	7.3	8.4	6.1	8.4	7.5
	June 10	7.9	8.8	7.0	9.1	8.2
	June 30	9.5	9.3	9.3	9.2	9.4
	Mean	7.8	8.9	7.5	8.8	8.2
Muju	April 30	8.0	9.0	6.8	8.6	8.1
	May 20	8.4	9.0	7.5	9.3	8.5
	June 10	8.9	9.8	9.1	10.1	9.5
	June 30	10.3	9.9	10.8	10.5	10.4
	Mean	8.9	9.4	8.6	9.6	9.1

Table 17. Significance analysis of seeding date, variety and planting region on C18:3 contents of soybean seed. (Unit: %)

Source	Seeding date		Variety		Region	
F-value	15.38**		7.04**		15.57**	
Mean value	April 30	7.9c	Daewon	8.3ab	Miryang	8.2b
	May 20	8.0bc	Daepung	9.1a	Muju	9.1a
	June 10	8.8b	Daol	8.0b	-	-
	June 30	9.8a	Cheongja3	9.1a	-	-

Table 18. Isoflavones contents of seed as affected by seeding dates and cultivated regions (Unit: mg/g)

Region	Seeding date	Variety				Mean
		Daewon	Daepung	Daol	Cheongja3	
Miryang	April 30	1.8	3.5	0.7	2.3	2.1c
	May 20	2.2	4.1	0.6	2.5	2.4bc
	June 10	2.8	4.8	0.8	3.2	2.9abc
	June 30	4.9	5.7	1.3	3.2	3.7a
	Mean	2.9	4.5	0.9	2.8	2.8
Muju	April 30	2.8	5.2	1.1	2.8	3.0abc
	May 20	3.1	4.8	1.2	3.0	3.0abc
	June 10	2.9	4.7	1.4	2.9	3.0abc
	June 30	3.2	5.0	1.6	3.3	3.3ab
	Mean	3.0	4.9	1.3	3.0	3.1
Mean		2.9b	4.7a	1.1c	2.9b	-

Table 19. Anthocyanin contents of Cheongja 3 seed coat, as affected by seeding dates and cultivated regions (Unit: mg/g)

Region	Seeding date				Mean
	4/30	5/20	6/10	6/30	
Miryang	9.1	14.1	14.5	13.6	12.8
Muju	18.1	18.6	22.7	19.4	19.7
Mean	13.7	16.4	18.6	16.5	-

Table 20. Significance analysis of anthocyanin content as affected by region, seeding year and seeding date (Unit: mg/g)

Source	Region		Year		Seeding date	
F-value	59.63**		8.7*		5.29*	
Mean value	Miryang	12.8b	2005	15.0b	April 30	13.7b
	Muju	19.7a	2006	17.6a	May 20	16.4ab
	-	-	-	-	June 10	18.6a
	-	-	-	-	June 30	16.5ab

becomes lower. Highest anthocyanin content was recorded from the June 30 seeding. These findings agreed with Ubi *et al.*'s report (2006) that anthocyanin synthesis was higher in relatively cool temperature than in high temperature.

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