Analysis of Genotypic Variation in Photo- and Thermo-sensitivities in Soybean (*Glycine max* (L.) Merrill) Adaptable to Tropical Areas

（熱帯地域に適するダイズの日長及び温度に対する感受性の品種間差異の解析）

MD. RASADUL ISLAM

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DISSERTATION

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Symbols and Abbreviations

ATEF	Accumulated temperature from emergence to first flower open
$ATEF_{ES}$	Accumulated temperature from emergence to first flower open in early sowing
$ATEF_{LS}$	Accumulated temperature from emergence to first flower open in late sowing
$ATEF_{13h}$	Accumulated temperature from emergence to first flower open under 13 h photoperiod
$ATEF_{10h}$	Accumulated temperature from emergence to first flower open under 10 h photoperiod
d	Days
DEF	Days from emergence to first flower open
DEF_{ES}	Days from emergence to first flower open in early sowing
DEF_{LS}	Days from emergence to first flower open in late sowing
h	Hours
IPF	Index of photosensitivity of flowering
GmWMC	world soybean mini-core collections
JGP	Juvenile growth phase
RJGP	Relative JGP

Chapter 1

General Introduction

Soybean (*Glycine max* (L.) Merrill) is an important commercial crop. It has high nutritional values, briefly 100 grams of mature soybean seeds contain 36.49 g of protein, 19.94 g fat, 30.16 g carbohydrates, 7.33 g sugar, 9.3 g dietary fiber, 446 kcal energy (Fig. 1-1) according to nutrient database of USDA (2016).

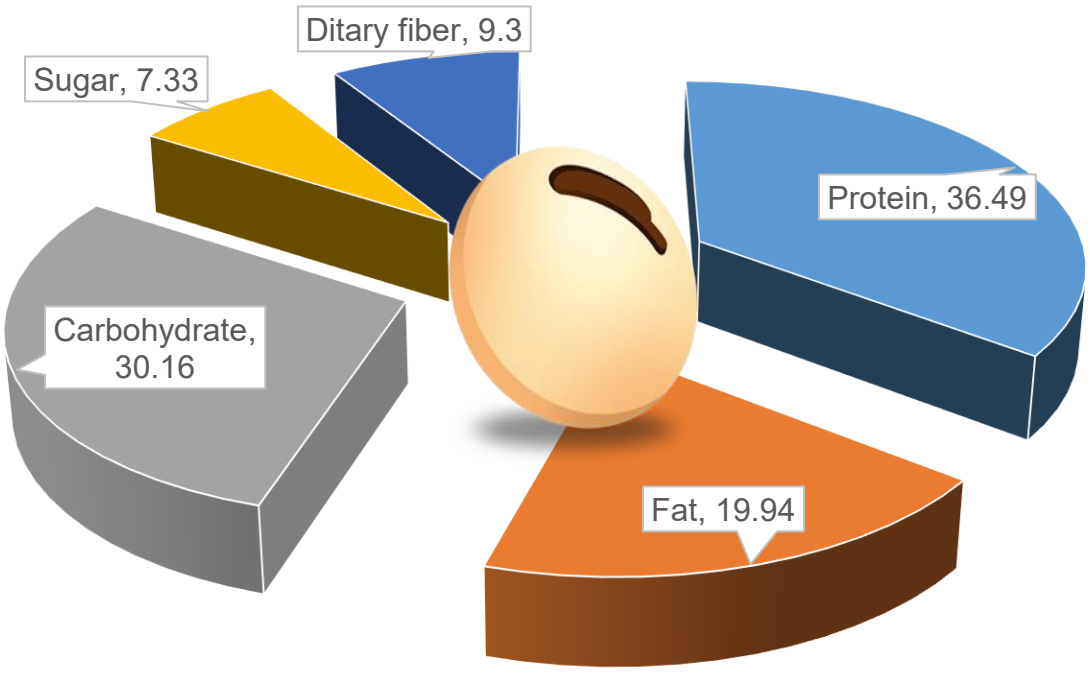

Fig. 1-1. Nutritional value per 100 g of mature soybean seed.

Source: USDA, 2016

Besides, soybeans are an exceptional source of vitamins, minerals and some functional elements of human body for instance isoflavones, lecithin and polysaccharide. It is important as cow feed (soybean meal and roasted soybean) to rise the meat and milk production as well as it is an ingredient of poultry and fish feed. It is also used to make industrial materials, i.e. biodiesel, printing ink, waxes, lubricant, fiber and textile, and adhesive. Thus, soybean has become a major goods for the world trade market (Sonka et al., 2004).

Linguistic, geographical, and historical evidence recommended that soybean is originated in China (Li et al., 2008). However, the United States (117.2 million of tons), Brazil (96.3 million of tons) and Argentina (58.8 million of tons) are the world's largest soybean producers (Fig. 1-2) and represent more than 80% of global soybean production (FAO, 2016).

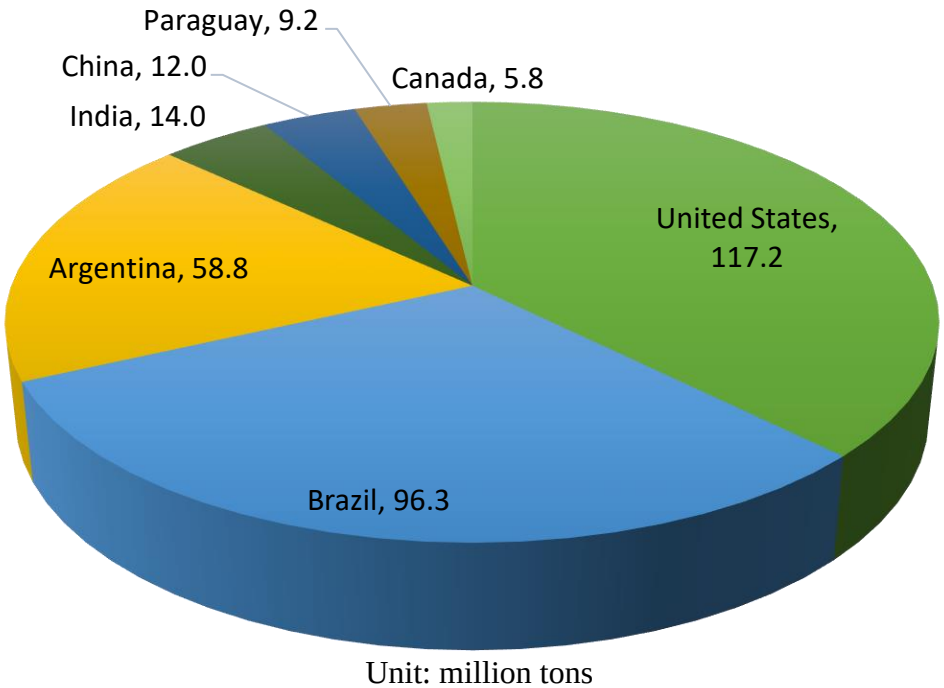

Fig. 1-2. Major soybean production countries of the world in 2016.

Source: FAO, 2016.

It is worthwhile noting that the demand for soybean in tropical Asia as a major protein resource for food, feed, and industrial uses has been increasing gradually. However, the self-sufficiency in most Asian countries are 40% in Bangladesh, 35% in Indonesia, 16% in China, 11% in Korea, 11% in Vietnam, 8% in Japan, and 6% in Thailand, which is inadequate to meet their high demand (Fig. 1-3). Therefore, it is necessary to increase soybean production area and yield for keeping pace with growing demand in tropical Asia.

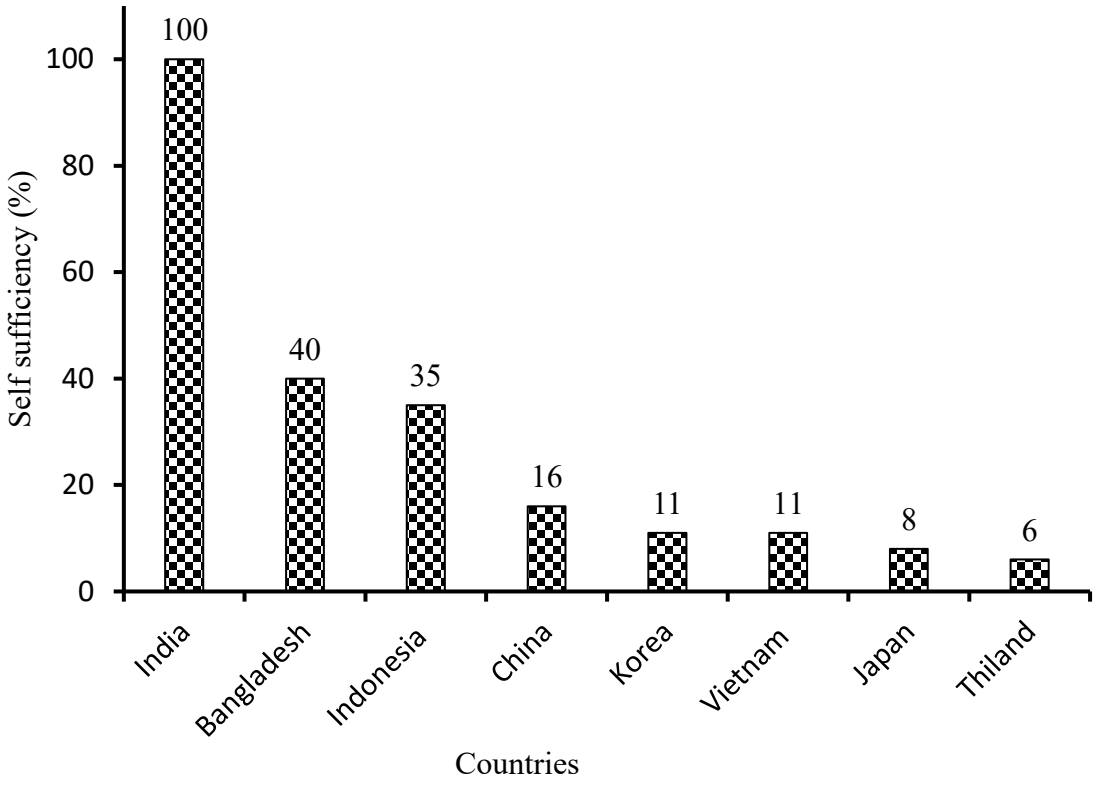

Fig. 1-3. Soybean self-sufficiency in some Asian countries.

Source: FAO, 2014.

In addition, seed yield is extremely low in some Asian countries (India, Indonesia, Vietnam) compared to Argentina, Brazil, USA (Fig. 1-4).

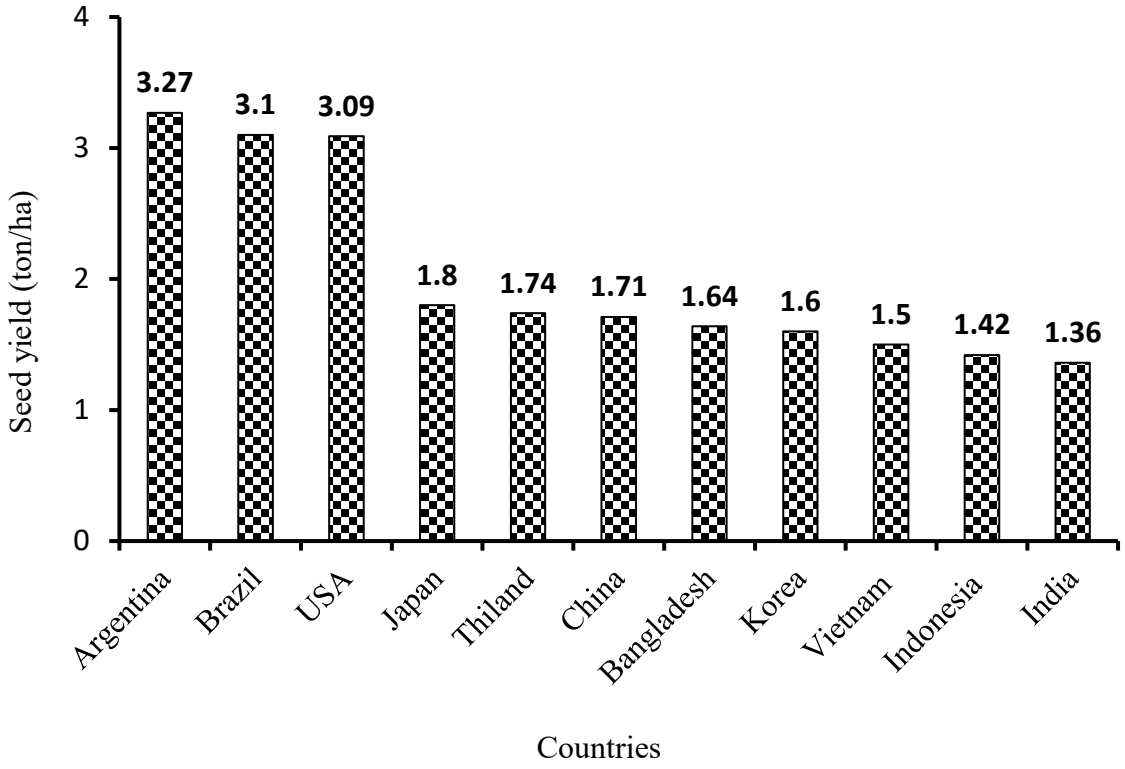

Fig. 1-4. Soybean seed yield in some countries.
Source: FAO 2016

The main reason of low seed yield in tropical areas could be insufficient vegetative growth caused by early flowering. Early flowering is generally brought by short photoperiod and high temperature in soybean (Board and Hall, 1984). Therefore, both short photoperiod and high temperature are prime yield limiting factors in tropical areas. In these case, strong photosensitive genotypes could be useful in tropical areas. Another major concern to soybean production in tropical areas is juvenile growth phase (JGP, being the period of growth which no flower initiation is possible). The genotypes with long JGP could maintain long vegetative growth even at short photoperiod (Fig. 1-5) and produce high seed yield in tropical areas.

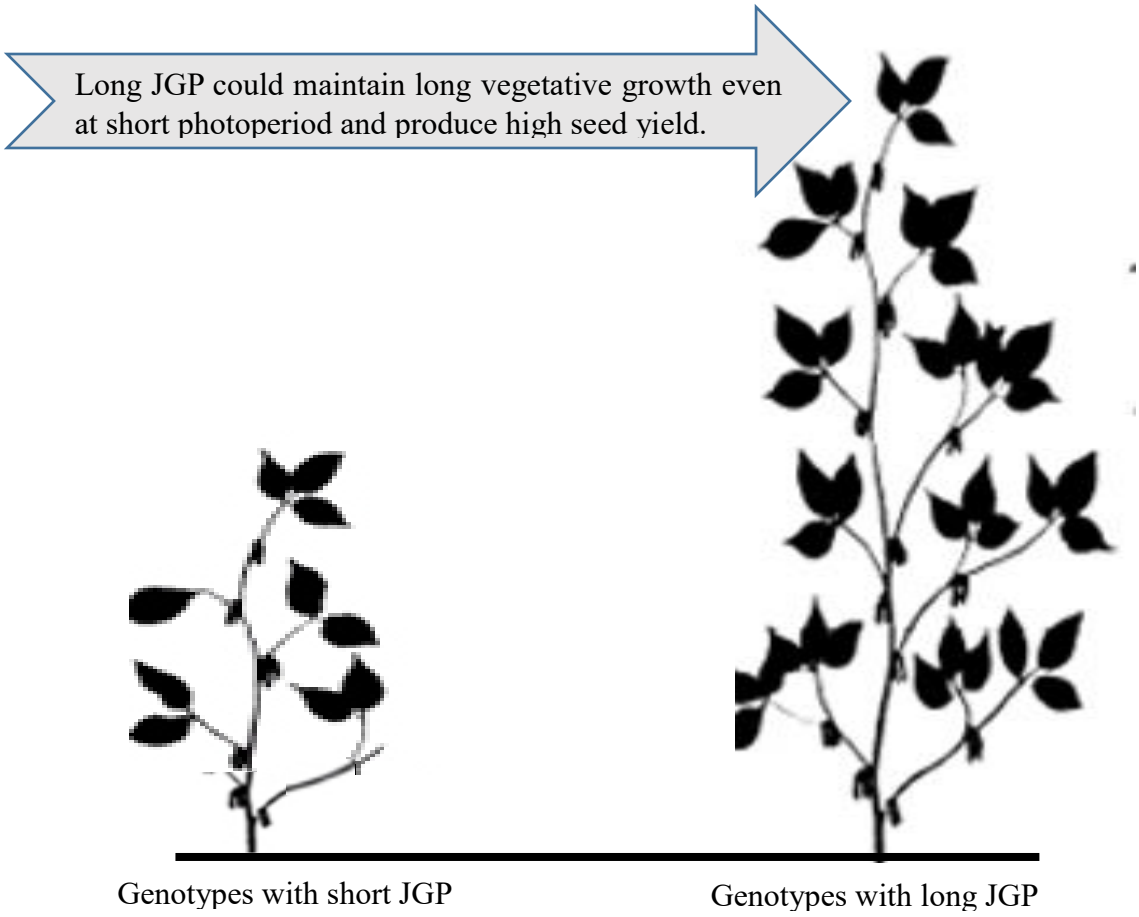

Fig. 1-5. Hypothetical sketch showing the influence of JGP on vegetative growth under short photoperiod.

Moreover, the less photosensitive and long JGP genotypes are extremely important to produce suitable genotypes for tropical areas through breeding programs. The better knowledge about the responses to photoperiod and temperature on soybean growth and development as well as identification of long JGP genotypes of soybean will be a key breakthrough in overcoming yield limiting problem in tropical areas.

Generally, degree of latitude affects photoperiod and consequently photoperiod influence soybean growth and development. Besides, photoperiod not only regulates the duration of flowering, but also affect the stage development after flowering. Previous reports showing that soybean development responds quantitatively to photoperiod after later stage of flowering (Grimm et al., 1994; Summerfield et al., 1998). Several studies indicated that photoperiod controls plant development and seed yield in soybean (Johnson et al., 1960; Mann and Jaworski, 1970; Raper and Thomas, 1978). As a result, a quantitative description of different growth stages and yield traits based on different photoperiod may allow selection of desirable genotypes for specific location.

On the other hand, temperature is another environmental factor which shows daily and seasonal fluctuations. These fluctuations in temperature also affect soybean plant growth and development. Many reports concerned about the effects of temperature on soybean flowering under natural or controlled environments (Major et al., 1975: Wang et al., 1987; Hadley et al., 1984; Hatfield and Prueger, 2015). Garner and Allard (1930) studied four soybean cultivars for eight years and concluded that summer temperatures below 25°C delayed flowering two to three days for 1°C in the average temperature. Steinberg and Garner (1936) reported that increasing temperature shortened flowering time in soybean up to an optimum temperature of 28°C, above which flowering time was delayed. Brown (1960) reported that growth and development of soybean plant stopped at temperature below 10°C and slowed at more than 30°C. It has also been documented that early maturing genotypes are more sensitive to temperature rather than photoperiod (Champman, 1986). However, it is not clear if temperature just accelerate the growth stage or trigger the flower initiation.

Although soybean is originated in temperate region, nowadays it is grown widely from low to high latitude regions. Broad growing condition has been facilitated by the identification of different level of photosensitive and JGP genotypes. For example, Low photosensitive genotypes could be grown comprehensively from low to high latitude areas. Additionally, long JGP extend vegetative phase and improve seed yield in tropical areas. Integration of long JGP into soybean breeding program is leading to improvement in soybean production in low latitude area. Brazilian researchers first introduced the long JGP and subsequently enabled step-up of soybean production to regions below 15˚ latitude (Neumaier and James, 1993; Destro et. al., 2001).

While more attention has been paid to the photosensitivity and JGP for the expansion of soybean production, particularly in tropical areas, very little is known about the JGP estimation in a physiological way and its relationship with photosensitivity.

As reported facts, this study was undertaken to provide crucial information for the enhancement of soybean production in tropical areas. To achieve this purpose, we have discussed about the genotypic variation of response to photoperiod in different growth stages and seed yield in the soybean world mini-core collections (*GmWMC*) in Chapter 2. Then, we have discussed about the flowering response of the soybean plant to temperature in chapter 3. Afterward, we provided useful technique to evaluate the photosensitivity and JGP and necessary information for the selection of adaptable genotypes, especially for tropical areas in chapter 4. Finally, a general discussion is exhibited in Chapter 5.

Chapter 2

Variation of Response to Photoperiods in Different Growth Stages and Seed Yield in *GmWMC* Genotypes

1. Introduction

The demand for soybean has been increasing as a result of growing populations and rising incomes, particularly in tropical Asia. Soybean production in those areas face many problems, including short photoperiod, drought, and high temperature. The growth period is shortened with short photoperiod and high temperature in soybean due to earlier flowering time. This phenomenon reduces the vegetative growth (Egli et al., 1987) and produces lower seed yield (Boerma and Ashley, 1982). This is a major problem in soybean production in tropical areas. However, Fatichin et al. (2013) reported that seed yield in late sowing (short photoperiod and high temperature) could be increased by choosing of adaptive genotypes based on larger seed number and/or longer seed filling periods. This implies that soybean seed yield reduction by late sowing could be overcome by proper genotype selection. A better concept is needed about yield-limiting factors, i.e. various yield components and time of different growth stages, which are affected by late sowing.

Photoperiod sensitivity limits farmers to select suitable genotypes, determine the best planting date, and predict seed yield as well as to identify areas that are generally adaptable in soybean. Moreover, several reports indicated the existence of photoperiod-insensitive genotypes based on flowering dates by changing photoperiod in a controlled environment (Criswell and Hume, 1972; Huxley et al., 1974; Cregan and Hartwing, 1984). Photoperiod-insensitive genotypes help to expand soybean production in tropical areas.

Shanmugasundaram and Tsou (1978) reported that photoperiod controls plant size, dry matter production, and seed yield potentiality in soybean. In addition, photoperiod regulates the duration of most development phases of soybean (Raper and Kramer, 1987). In particular, post flowering phases are considered extremely important for soybean seed yield production. Nico et al. (2016) reported photoperiod extension delayed the duration from flowering to pod elongation. It is also reported that short photoperiod and high temperature stimulated pod formation in soybean (Thomas and Raper, 1976; Raper and Thomas, 1978). Han and Wang (1995) reported that the responses to photoperiod after flowering in soybean existed with different growth stages. Besides, several other aspects of soybean are also influenced by photoperiod such as vegetative growth, dry matter partitioning towards pods and seeds. However, less information is known about genotypic analysis in the response to photoperiod on post flowering stages in soybean.

From this background, there is a major impact on the pattern of responses to photoperiod in soybean growth, development and yield productivity. Thus, a systematic investigation is needed for better understanding of genotypic variations in the response of soybean growth development to photoperiod. Therefore, the performance of plant growth development and seed yield was tested under field condition by changing sowing time.

2. Materials and methods

Experimental design

The experiments were conducted during 2015 to 2017 in the experimental field at Saga University, Saga of Japan (33° 14' 32" N and 130° 17' 28" E). The plants were grown in the field at early sowing (long photoperiod) and late sowing (short photoperiod).

Plant materials and growth conditions

Eighty-two genotypes of the soybean world mini-core collections (*GmWMC*) provided by the NARO gene bank of Japan were used as plant materials in Table 2-1 (Kaga, et al., 2012). The collections consist of a wide variation in the origin, maturing earliness, and some other growth habits. The seed color and size were shown in Fig. 2-1. The seeds were sown on 28 May (2015), 23 May (2016) and 24 May (2017) for early sowing, and 4 August (2015 and 2017) and 5 August (2016) for late sowing. The plants would be exposed to long photoperiod in early sowing, whereas to short photoperiod in late sowing. The contents of early and late sowing were shown in Fig. 2-2 and Fig. 2-3. Five seeds were sown in each hill (5 hills / genotype), which were arranged at 20 cm intervals with 70 cm row spacing. A basal chemical fertilizer, at a rate of $N : P_2O_5 : K_2O = 3 : 10 : 10$ g m^{-2}, and agricultural lime (100 gm^{-2}) were applied before plowing. Seedlings were thinned into two plants per hill when the first trifoliate leaf appeared. Weeding was done by hand tractor or hand and pesticide was applied when necessary.

Fig. 2-1. Seed color and size of the soybean world mini-core collections *(GmWMC)*.

Fig. 2-2. The contents of early sowing in 2016.

Fig. 2-3. The contents of late sowing in 2016.

Measurement and data collection

Photoperiod and temperature

Daily photoperiodic hours were recorded from sunrise to sunset according to Saga weather station; and 30 min each was added before sunrise and after sunset. Daily average temperature was measured at a standard weather station located approximately 100 m from the experimental field.

Phenological stages

The date of emergence (50% of plants with cotyledons above soil surface), first flowering (50% of plants with one flower at any node, R1), first pod appearance (Pod 5 mm long on one of the four uppermost nodes on the main stem, R3), the start of seed filling (Seed 3 mm long in a pod on one of the four uppermost nodes on the main stem, R5), and beginning maturity (one pod anywhere with its mature color, R7) were determined according to Fehr et al. (1971), contents of these stages were shown in Fig. 2-4 and Fig. 2-5. Additionally, node number for randomly selected three plants were recorded at first flowering stage.

Emergence

First flowering (R1)

Pod appearance (R3)

Start of seed filling (R5)

Fig. 2-4. The contents of emergence, pod appearance, and start of seed filling in soybean.

Beginning maturity (R7)

Full maturity (R8)

Fig. 2-5. The contents of beginning maturity and full maturity in soybean.

Measurement of yield component at full maturity (R8)

Stem height, node number in main stem, total node number plant^{-1}, number of branches, stem weight, and seed weight plant^{-1} were recorded for five randomly selected plants under late sowing in 2015.

Statistical analysis

The correlations with significant level were measured by using data analysis tool of Microsoft Excel (version 2016).

3. Results

Daily photoperiodic hours and mean air temperature for the entire growing season in the field experiment over 3 years are shown in Fig. 2-6. The average values of photoperiod and temperature were 15.13 h and 25.7°C in early sowing and 14.08 h and 28.2°C in late sowing from first emergence to the average date of flower open over 3 years in the field experiments.

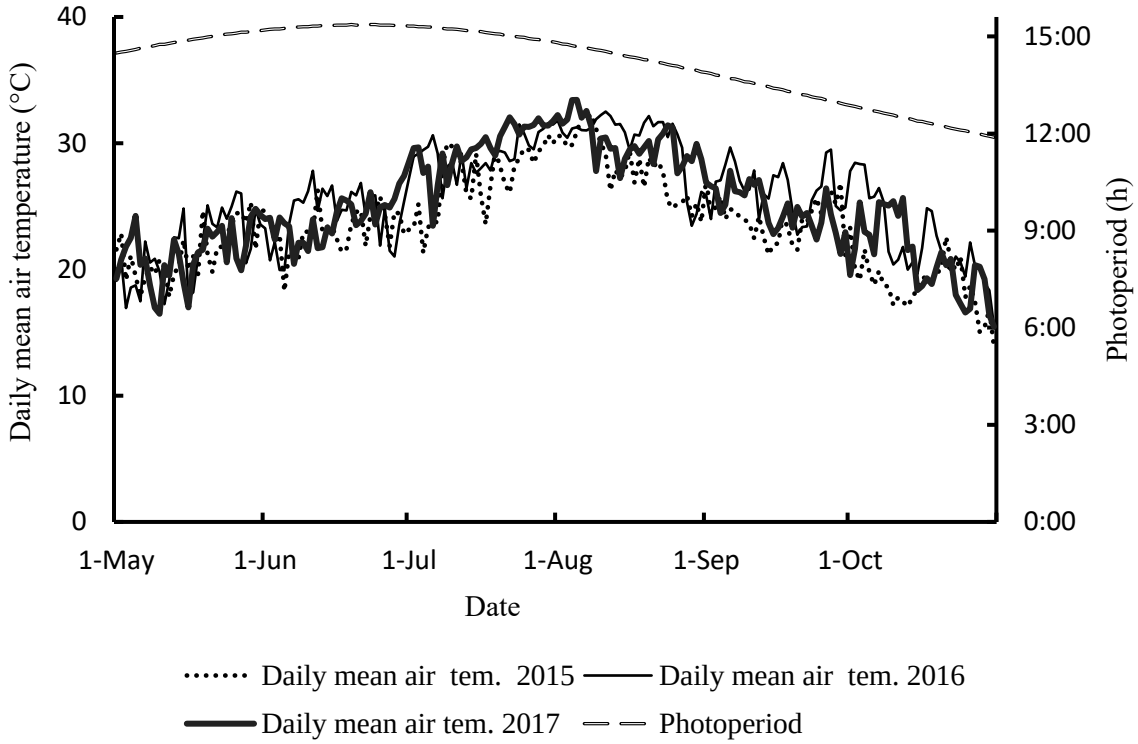

Fig. 2-6. Daily photoperiod and mean air temperature during experiment time over three years.

Source: http://www.nao.ac.jp/

Table 2-1 shows the variation in origin, DEF, days of R1−R3, R3−R5, and R5−R7 in early and late sowing as well as seed weight plant^{-1} in late sowing among the *GmWMC* genotypes. The DEF differed greatly from 23–92 d in early sowing, whereas it was 19–63 d in late sowing. The days of R1−R3 were 7−48 d in early sowing but 6−15 d in late sowing. In addition, the days of R3−R5 were 5−15 d in early sowing and 5−10 d in late sowing, whereas days of R5−R7 were 22−77 d in early sowing and 13−40 d in late sowing. The seed weight plant^{-1} differed greatly from 1.4–39.5 g. More than 2/3 genotypes provided poor seed weight plant^{-1} (< 20g plant^{-1}), whereas 16 genotypes produced 20−39.5 g seed weight plant^{-1}. Besides, there was no relation between origin of genotypes and seed weight plant^{-1} (Table 2-1).

Table 2-1. duration of different growth stages and seed weight plant^{-1} (SEW) in *GmWMC*.

ID number	Genotypes	Origin	DEF_{ES} (d)	DEF_{LS} (d)	R1-R3 $_{ES}$ (d)	$R1\text{-}R3_{LS}$ (d)	$R3\text{-}R5_{ES}$ (d)	$R3\text{-}R5_{LS}$ (d)	$R5\text{-}R7_{ES}$ (d)	$R5\text{−}R7_{LS}$ (d)	SEW (g)
*GmWMC*001	Fiskeby V	Sweden	23	19	8	6	5	5	60	27	5.4
*GmWMC*006	Ks 1034	Malaysia	32	23	13	8	10	5	57	28	9.3
*GmWMC*011	Seita	Rep. Korea	40	25	26	9	5	5	50	40	6.4
*GmWMC*012	Manshuu	China	37	25	14	7	8	5	51	30	5.8
*GmWMC*014	Kls 203	Rep. Korea	78	40	20	12	6	5	29	25	11.8
*GmWMC*015	Chuuhoku 2	Rep. Korea	43	25	19	8	11	5	37	29	5.8
*GmWMC*018	Rigai Seitou	China	73	39	23	10	7	6	27	18	15.3
*GmWMC*019	Chousenshu (Cha)	Korea	30	20	9	8	15	6	46	21	3.4
*GmWMC*020	Pochal	Taiwan	47	28	14	8	9	10	52	32	16.2
*GmWMC*022	Nezumi Meta	Korean Peninsula	77	37	19	10	9	5	36	28	-
*GmWMC*024	Chieneum Kong	Rep. Korea	41	26	15	6	7	5	58	29	3.8
*GmWMC*027	Kongnamul Kong	Rep. Korea	43	26	19	7	11	5	48	29	9.3
*GmWMC*029	Shirosota	korean Peninsula	40	27	13	7	7	5	55	25	15.3
*GmWMC*035	Pekin Dai Outou	China	34	21	7	7	8	5	-	31	5.9
*GmWMC*036	Masshokutou (Kou 502)	China	30	21	9	7	9	5	-	28	1.4
*GmWMC*038	Ichiguuhou	China	78	36	18	9	6	7	34	19	17.8
*GmWMC*042	Masshokutou (Kou 503)	China	30	22	9	7	15	5	-	21	3.9
*GmWMC*045	Okjo	Rep. Korea	45	26	13	10	9	6	45	35	15.1
*GmWMC*046	Ke 32	Philippines	24	20	10	7	10	5	64	23	7.8
*GmWMC*048	Heamnam	Rep. Korea	78	36	20	10	6	6	45	21	18.0
*GmWMC*066	Heukdaelip	Rep. Korea	31	23	15	10	10	7	60	33	10.7
*GmWMC*070	Choyoutou	China	28	22	16	9	8	6	-	-	-
*GmWMC*071	Pk 73-54	India	48	27	31	9	9	7	52	40	8.9
*GmWMC*072	M 581	India	56	31	24	10	7	5	37	26	14.9
*GmWMC*073	Uronkon	Korean Peninsula	38	26	23	8	7	7	46	39	8.8
*GmWMC*075	Cheongye Myongtae	Rep. Korea	37	23	15	6	9	7	44	31	8.9
*GmWMC*083	Keumdu	Rep. Korea	39	28	14	7	8	5	52	34	6.0
*GmWMC*084	Peking	China	46	26	14	8	8	5	39	26	4.5
*GmWMC*086	Anto Shoukokutou	China	26	20	11	7	9	5	-	24	5.4

ID number	Genotypes	Origin	DEF_{ES} (d)	DEF_{LS} (d)	R1-$R3_{ES}$ (d)	R1-$R3_{LS}$ (d)	R3-$R5_{ES}$ (d)	R3-$R5_{LS}$ (d)	R5-$R7_{ES}$ (d)	R5−$R7_{LS}$ (d)	SEW (g)
*GmWMC*089	Bongchunbaekjam	China	37	24	11	7	9	6	47	28	14.9
*GmWMC*094	Jeokgak	Rep. Korea	50	30	22	9	5	6	39	25	15.7
*GmWMC*103	Senyoutou	China	78	40	24	14	6	5	33	20	19.1
*GmWMC*107	Hakka Zashi	China	29	22	12	6	7	6	-	21	7.7
*GmWMC*108	Karasumame	China	41	29	18	8	9	10	25	21	17.0
*GmWMC*113	Baritou 3 A	Indonesia	43	28	11	6	8	7	37	31	16.4
*GmWMC*115	Williams 82	USA	31	24	27	8	5	6	38	24	19.1
*GmWMC*118	Oudu	Rep. Korea	31	22	20	10	10	6	57	32	5.2
*GmWMC*119	Hakubi	China	33	24	15	9	8	5	48	29	14.4
*GmWMC*120	U 1416	Nepal	62	32	25	7	-	6	47	31	7.1
*GmWMC*122	Gapsanjaelae(I)	Rep. Korea	34	23	24	11	8	6	41	24	15.7
*GmWMC*123	N 2295	Nepal	65	32	30	10	7	5	46	29	19.4
*GmWMC*125	Bhatmas	Nepal	67	33	31	10	6	5	46	29	20.0
*GmWMC*129	Aoki Mame	China	74	35	28	9	6	6	47	34	12.5
*GmWMC*132	L 2a	Philippines	41	29	17	8	9	5	43	24	27.1
*GmWMC*136	Local Var (Seputih Raman)	Indonesia (Sumatra)	83	43	21	9	6	6	29	13	22.6
*GmWMC*138	Col/Pak/1989/Ibpgr/2326(1)	Pakistan	60	28	32	9	6	7	49	27	16.8
*GmWMC*141	Petek	Indonesia	71	38	24	10	6	10	26	18	15.2
*GmWMC*142	Java 5	Indonesia	88	48	19	9	9	7	25	24	24.9
*GmWMC*143	M 44	India	65	32	28	9	6	5	37	29	13.8
*GmWMC*144	M 918	India	77	36	20	10	7	5	35	31	24.7
*GmWMC*146	Hm 39	India	69	35	28	11	8	6	38	31	19.4
*GmWMC*147	Col/Thai/1986/Thai-78	Thailand	58	35	28	11	11	6	39	32	22.9
*GmWMC*148	M 42	India	88	36	22	11	5	6	34	33	-
*GmWMC*150	U 1042-1	Nepal	76	35	33	13	-	7	27	31	6.5
*GmWMC*151	Java 7	Indonesia	79	35	19	11	6	6	30	25	13.8
*GmWMC*152	U 1290-1	Nepal	74	37	24	9	7	6	39	22	24.4
*GmWMC*154	Manshuu Masshokutou	China	66	35	23	11	9	5	40	25	25.5
*GmWMC*156	U 8006-3	Nepal	60	33	48	13	5	7	36	24	10.6
*GmWMC*159	Col/Pak/1989/Ibpgr/2323(2)	Pakistan	49	24	36	12	6	7	41	27	8.2
*GmWMC*160	N 2392	Nepal	-	50	-	14	-	8	-	22	5.6
*GmWMC*162	Col/Thai/1986/Thai-80	Thailand	57	37	32	12	10	7	50	31	21.8
*GmWMC*163	N 2491	Nepal	91	47	21	11	5	10	37	34	23.5

ID number	Genotypes	Origin	DEF_{ES} (d)	DEF_{LS} (d)	R1-R3 $_{ES}$ (d)	$R1-R3_{LS}$ (d)	$R3-R5_{ES}$ (d)	$R3-R5_{LS}$ (d)	$R5-R7_{ES}$ (d)	$R5-R7_{LS}$ (d)	SEW (g)
*GmWMC*165	Karasumame (Shinchiku)	Taiwan	41	31	15	9	9	6	55	21	14.1
*GmWMC*166	Merapi	Indonesia (Sumatra)	64	41	25	14	8	5	35	22	16.2
*GmWMC*168	L 317	India	80	42	16	11	9	5	33	23	20.9
*GmWMC*169	Hakuchikou	China	31	22	16	7	8	6	77	30	9.4
*GmWMC*170	M 652	India	70	37	36	10	5	6	30	30	6.8
*GmWMC*171	U-1741-2-2 No.3	Nepal	44	32	36	13	6	7	44	25	15.8
*GmWMC*173	Karasumame (Naihou)	Taiwan	74	51	21	10	7	6	33	36	39.5
*GmWMC*175	Bishuu Daizu	China	63	32	24	9	-	5	42	26	19.3
*GmWMC*176	Sandek Sieng	Cambodia	92	47	23	10	7	5	23	26	14.9
*GmWMC*181	Chiengmai Palmetto	Thailand	67	35	29	12	6	5	34	18	28.8
*GmWMC*182	Local Var. (Tegineneng) Purple Flower	Indonesia (Sumatra)	86	48	18	9	11	6	32	23	14.2
*GmWMC*182	Local Var. (Tegineneng) White Flower	Indonesia (Sumatra)	72	39	17	11	11	6	40	30	25.7
*GmWMC*183	Karasumame (Heitou) Yellow Seed	Taiwan	41	27	14	8	9	5	44	29	9.8
*GmWMC*183	Karasumame (Heitou) Black Seed	Taiwan	44	31	14	9	10	9	34	22	10.5
*GmWMC*186	Ringgit	Indonesia (Sumatra)	72	45	16	9	9	6	39	21	15.8
*GmWMC*187	Kadi Bhatto	Nepal	83	38	22	14	5	6	28	-	8.5
*GmWMC*188	E C 112828	India	84	45	19	11	6	7	22	23	23.0
*GmWMC*190	San Sai	Thailand	84	51	18	6	6	6	24	-	21.5
*GmWMC*191	Miss 33 Dixi	Philippines	85	63	25	10	5	5	-	-	-
*GmWMC*192	U 1155-4	Nepal	61	38	41	15	-	6	34	20	19.8

DEF_{ES} and DEF_{LS} are the days from emergence to first flower open in early and late sowing; $R1-R3_{ES}$ and $R1-R3_{LS}$ are the days from R1 to R3 in early and late sowing; $R3-R5_{ES}$ and $R3-R5_{LS}$ are the days from R3 to R5 in early and late sowing; $R5-R7_{ES}$ and $R5-R7_{LS}$ are the days from R5 to R7 in early and late sowing. The genotypes are arranged in based on ID number. The values of DEF were the average of three years; the values of R1−R3 were the average of two years; the values of R3−R5 and R5−R7were from 2017 and 2015; and the values of seed weight plant^{-1} were from late sowing in 2015.

Fig. 2-7 shows the relationship of DEF between early and late sowing from 2015 to 2017. The relationship of DEF between early and late sowing was highly positive (r = 0.8755 (2015); r = 0.8826 (2016) and r = 0.8821 (2017); $p < 0.001$) and DEFs were shorter in late sowing in all genotypes in all years.

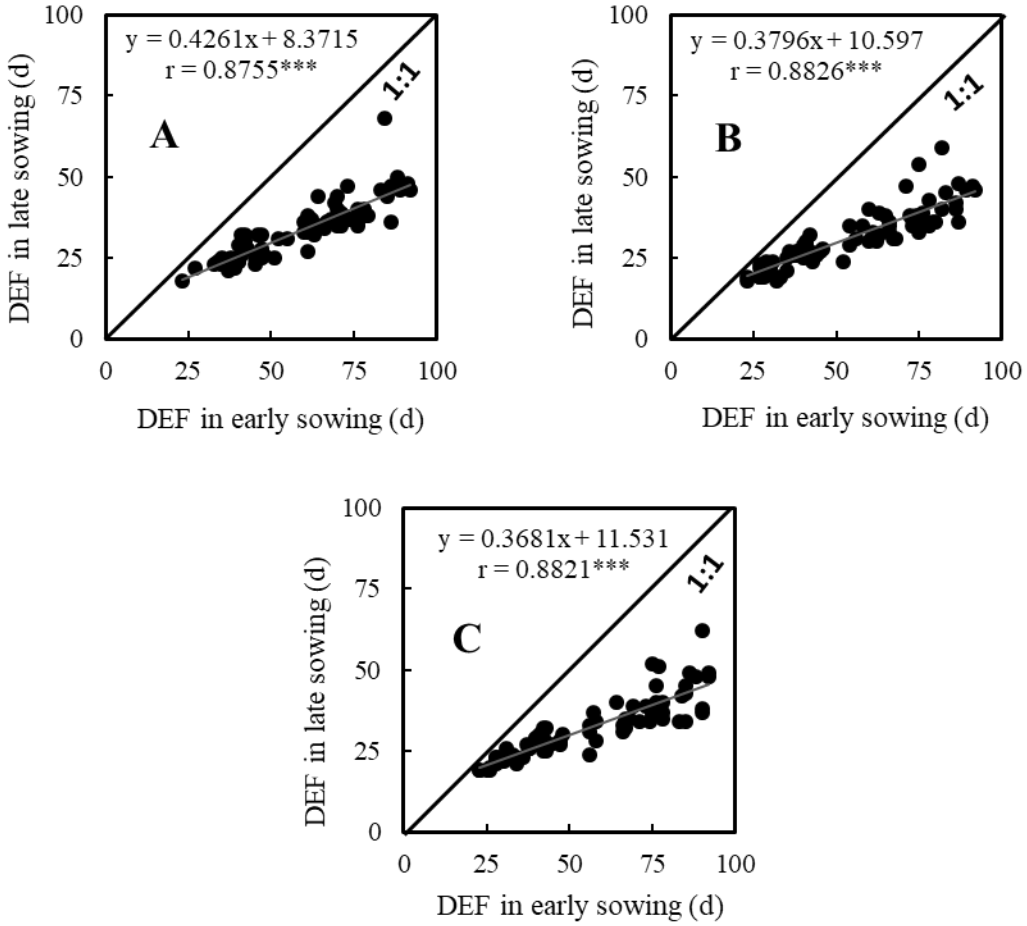

Fig. 2-7. Relationships of the days from emergence to first flower open (DEF) between early and late sowing in 2015 (A), 2016 (B) and 2017 (C). *** denotes significant at $P < 0.001$ and line represents the 1:1 ratio.

Fig. 2-8 shows the relationship in days of R1–R3 (pod formation) between early and late sowing. A significant positive relationship was found in the days of R1–R3 between early and late sowing in both 2016 ($r = 0.63$, $p < 0.001$) and 2017 ($r = 0.50$, $p < 0.001$). Besides days of R1–R3 were shorter in late sowing in most of the genotypes in both years.

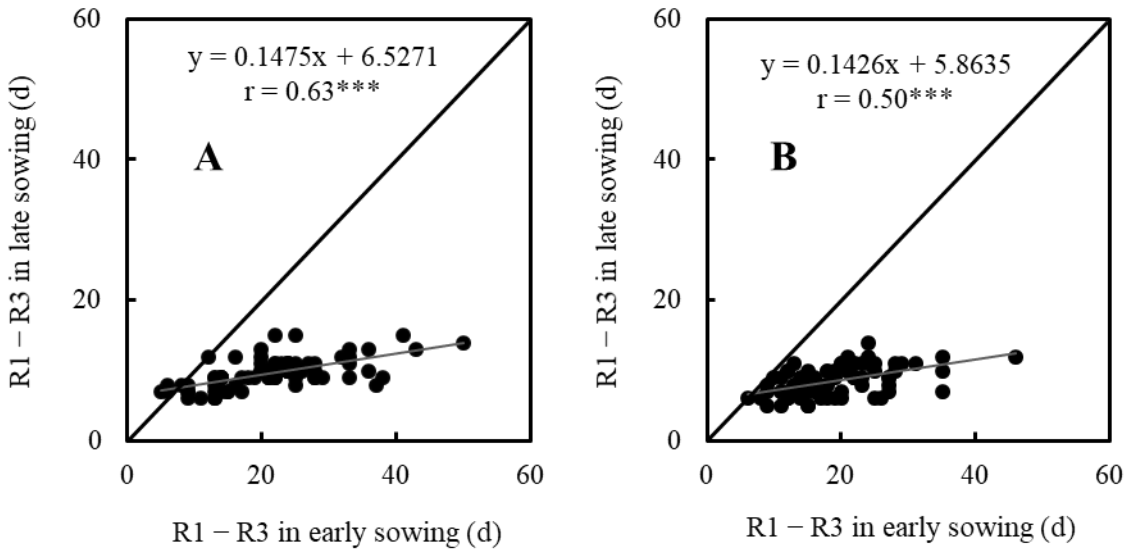

Fig. 2-8. Relationship between days of R1−R3 (duration for pod formation) in early and late sowing in 2016 (A) and 2017 (B). *** denotes significant at $P < 0.001$.

Fig. 2-9 shows the relationship in the days of R3–R5 (pod elongation) between early and late sowing. There was no significant relationship in days of R3–R5 between early and late sowing, which was contrary with that of days of R1–R3 (Fig. 2-9).

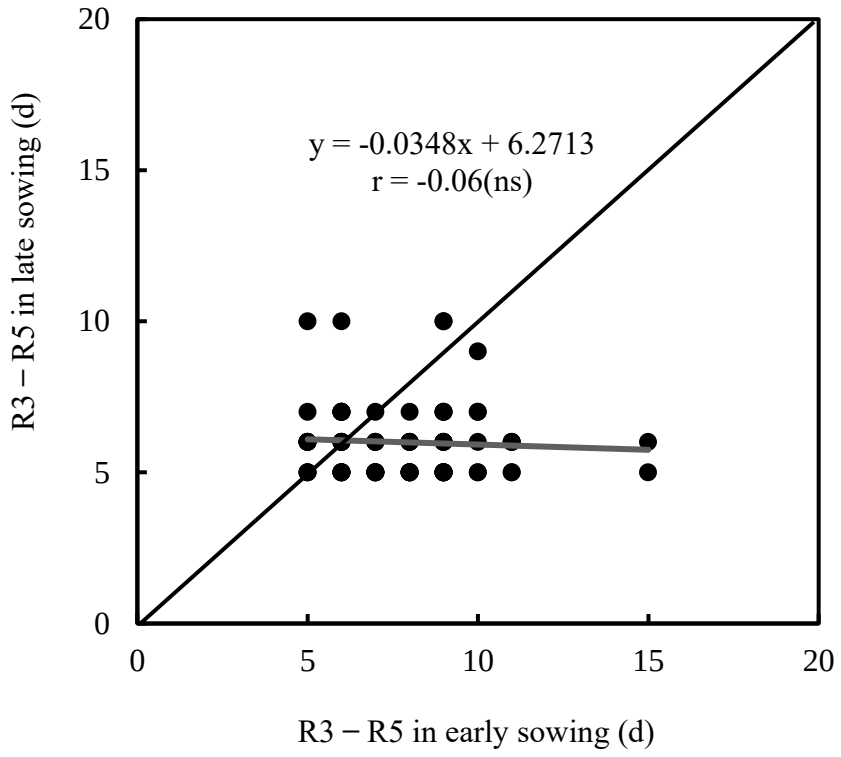

Fig. 2-9. Relationship between days of R3−R5 (duration for pod elongation) in early and late sowing in 2017. ns denotes not significant ($P > 0.05$) and *** denotes significant at $P < 0.001$. In this Figure some of the points overlapped.

Fig. 2-10 shows the relationship in the days of R5–R7 (seed filling) between early and late sowing. There was a significant positive relationship of the days of R5–R7 (seed filling) between early and late sowing ($r = 0.39$, $p < 0.001$) (Fig. 2-10).

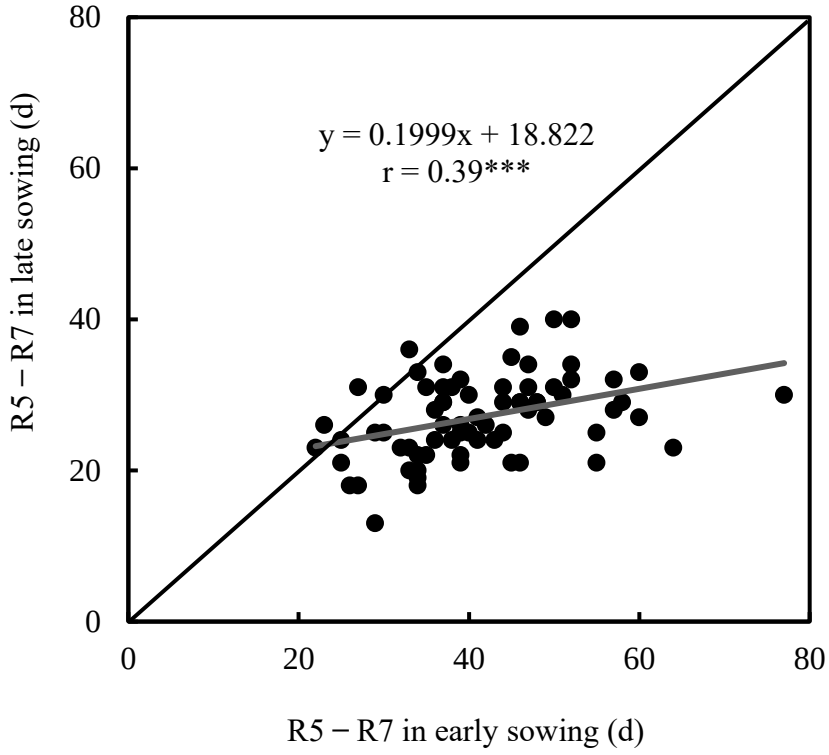

Fig. 2-10. Relationship between days of R5−R7 (duration of seed filling) in early and late sowing in 2015. ns denotes not significant ($P > 0.05$) and *** denotes significant at $P < 0.001$.

Fig. 2-11 shows the relationship between seed weight plant^{-1} and duration of different growth stages. The seed weight plant^{-1} was significantly correlated with DEF ($r = 0.61$, $p > 0.01$) and whole growing period ($r = 0.50$, $p > 0.01$) (Fig. 2-11 A and D), however was not correlated with duration from first flower open to start of seed filling ($r = 0.17$, $p > 0.05$) and seed filling period ($r = -0.12$, $p > 0.05$) (Fig. 2-11 B and C).

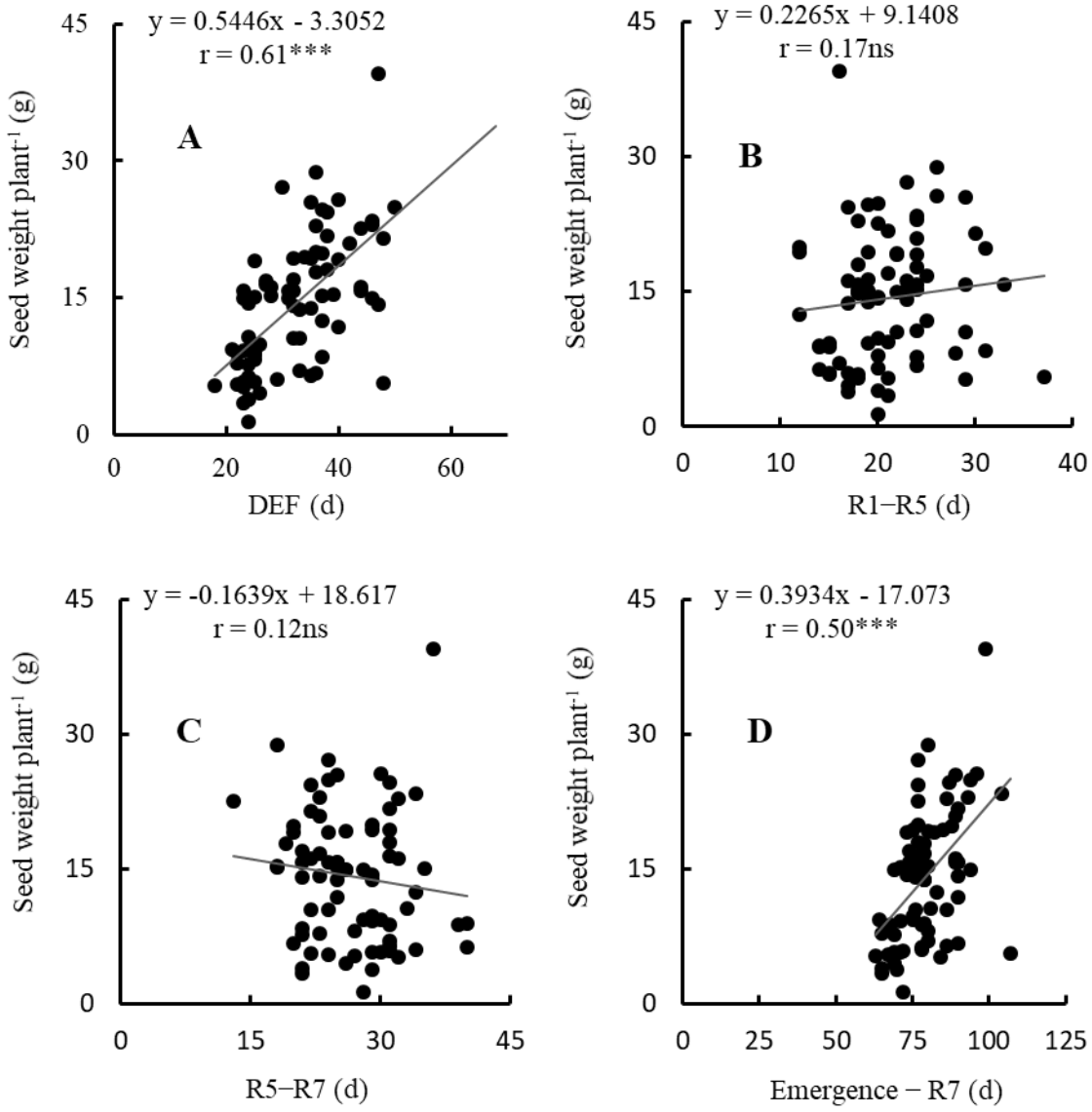

Fig. 2-11. Relationship between seed weight plant^{-1} and A. the days from emergence to first flower open (DEF); B. days from R1−R5 (first flower open to start of seed filling); C. days from R5−R7 (seed filling); D. days from emergence − R7 (whole growing period). *** and ns denote significant at $P < 0.001$ and not significant ($P > 0.05$), respectively. The data are the late sowing in 2015.

Fig. 2-12 shows the relationship between nodes number at flowering and seed weight plant^{-1}. Node number at flowering differed greatly from 7 to 15 and it showed positively correlation with seed weight plant^{-1} ($r = 0.49$, $p > 0.01$).

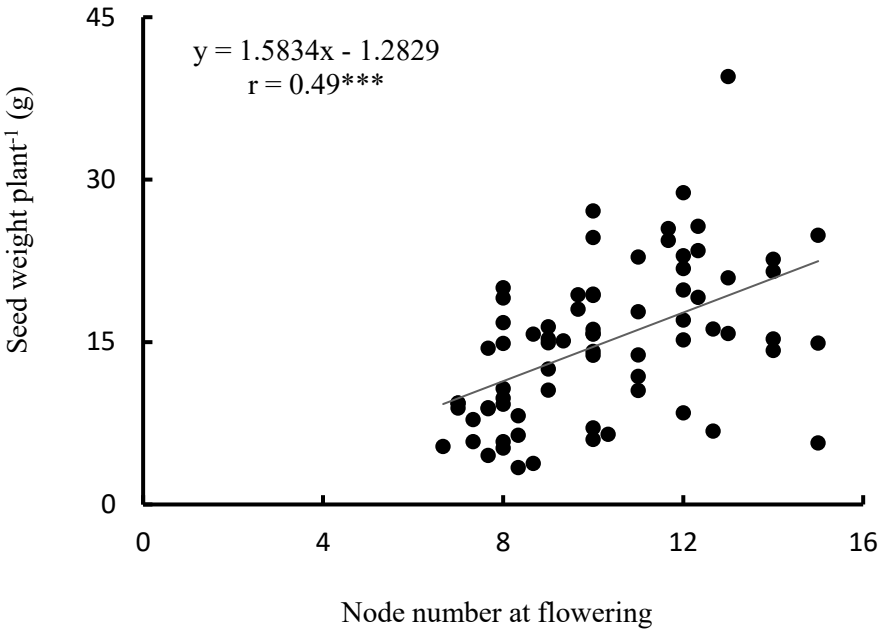

Fig. 2-12. Relationships between seed weight plant^{-1} and node number at flowering. *** denotes significant at $P < 0.001$. The data are the late sowing in 2015.

Fig. 2-13 shows the relationship between seed weight plant^{-1} and various yield relating parameters at R8 stage in soybean *GmWMC* genotypes. Seed weight plant^{-1} was positively correlated with total node number plant^{-1} ($r = 0.66$, $p < 0.001$), number of branch ($r = 0.59$, $p < 0.001$), node number in main stem ($r = 0.59$, $p < 0.001$), stem height ($r = 0.55$, $p < 0.001$), and stem weight ($r = 0.53$, $p < 0.001$), indicating seed weight was clearly related with vegetative growth. On the other hand, 100 seed weight was not associated with seed weight plant^{-1} ($r = 0.00$ns, $p > 0.05$).

Moreover, soybean genotypes such as Karasumame 'Naihou' (39.53 g plant^{-1}), Chiengmai Palmetto (28.80 g plant^{-1}), Local Var. 'Tegineneng' (25.68 g plant^{-1}), Manshuu Masshok (25.47 g plant^{-1}) and Java 5 (24.9 g plant^{-1}) produced higher seed weight plant^{-1} compared to the other genotypes (Table 2-1 and Fig. 2-13). These genotypes hold large total node number plant^{-1} such as Karasumame 'Naihou' (137), Chiengmai Palmetto (54), and Local Var. 'Tegineneng' (44), and Java (50) as well as hold longer DEF (Fig. 2-13 D).

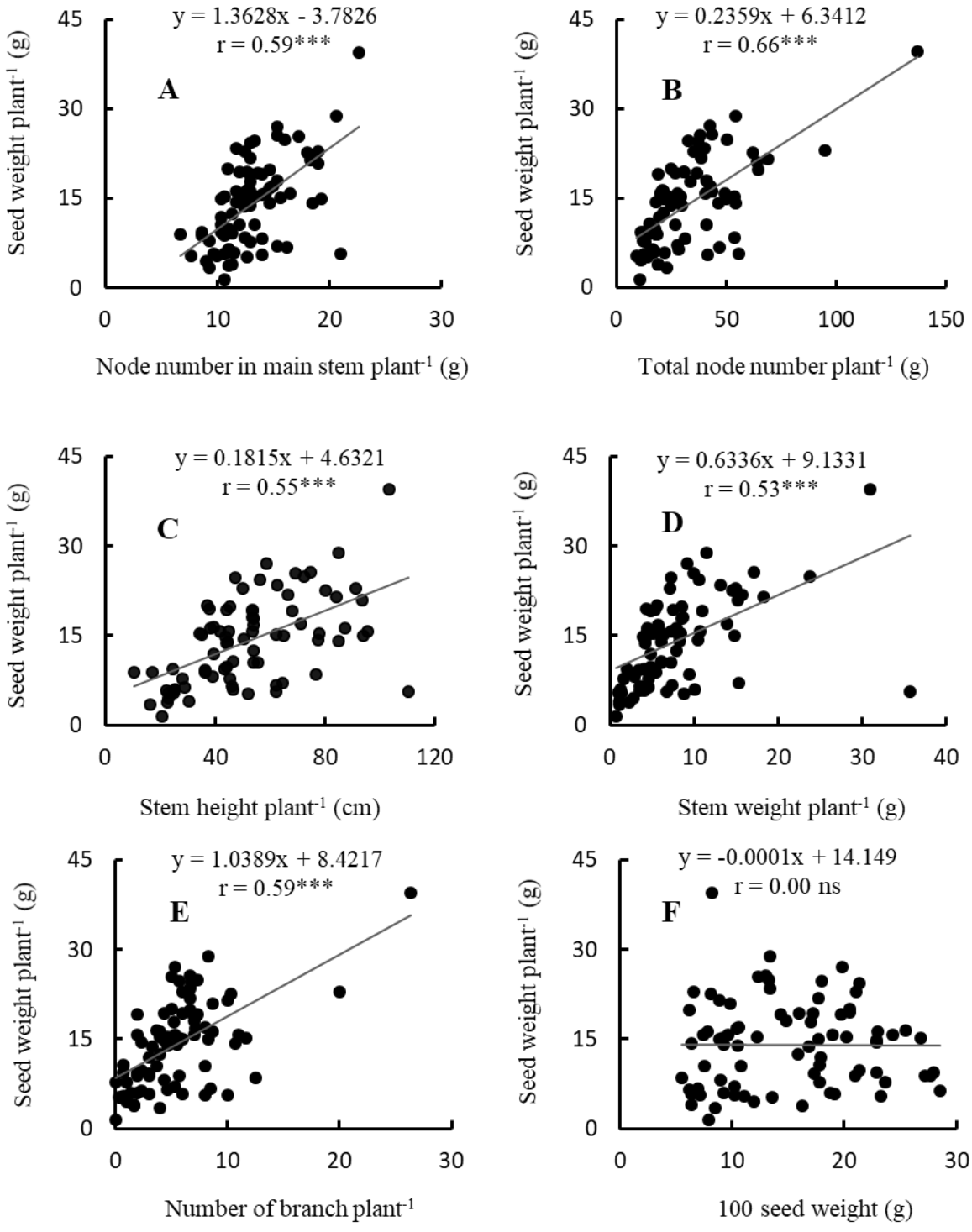

Fig. 2-13. Relationship between seed weight plant^{-1} and A. node number in main stem plant^{-1}; B. total node number plant^{-1}; C. stem height plant^{-1}; D. stem weight plant^{-1}; E. number of branch plant^{-1}; F. 100 seed weight. *** and ns denote significant at $P < 0.001$ and not significant ($P > 0.05$), respectively. The data are the late sowing in 2015.

4. Discussion

Understanding of photosensitivity in soybean provides an indication of the proper management to maximize yield potential and for wide geographic adaptability. Several studies indicated that photoperiod controls many aspects of soybean growth, such as plant development and yield (Johnson et al., 1960; Mann and Jaworski, 1970; Raper and Thomas, 1978). Board (2002) reported that a 29–276% increase in soybean seed yield through appropriate genotype selection based on vegetative growth for late sowing in Rouge, Louisiana, USA (30° N). Hence, there is a tremendous opportunity to improve soybean production in some regions by selecting appropriate genotypes based on sowing dates.

In this experiment, field data sets with sowing date treatments have obvious problem of photoperiod and temperature changing daily and interact each other. However, plants were exposed to longer photoperiod and lower temperature (15.13 h and 25.7°C) in early sowing, but shorter photoperiod and higher temperature (14.08 h and 28.2°C) in late sowing (Fig. 2-5). The variation in DEF were found to be larger in early sowing (23–92 d) than in late sowing (18–68 d) between 2015 and 2017 (Table 2-1). The reduction in DEF in late sowing might be caused by both the shorter photoperiod and higher temperature. Board and Hall (1984) reported that high temperature (27°C) shortened the flowering period compared with low temperature (21°C), particularly in short day condition. Additionally, in some early flowering genotypes in our study, the DEF were near to 1:1 line between early and late sowing (Fig. 2-7), indicating that the early flowering genotypes were less sensitive to

sowing time (photoperiod) compared with late flowering genotypes, which is similar with the indication of several reports (Cregan and Hartwing, 1984; Criswell and Hume, 1972; Polson,1972; Major 1975).

It is known that soybean plants respond to photoperiod even at the post flowering stage. Han et al. (2006) reported that pod appearance was delayed in long photoperiod condition. Zheng et al. (2003) showed that a lag period of pod growth (the period from flowering to the time a pod reached a length of 10 mm) in soybean was shortened by late sowing or short photoperiod. Heitholt et al. (1986) also observed notable variations in the duration of pod growth between genotypes in different photoperiodic conditions. Our result showed great variation in days of R1–R3 in early sowing, whereas there was less variation in days of R1–R3 in late sowing (Fig. 2-8), indicating that late sowing (short photoperiod) hastened pod appearance. Similarly, Khaliliaqdam (2014) stated that the duration of pod appearance decreased along with a decrease in photoperiod. In order to confirm the longer days of R1–R3 in early sowing, we set tags on the early opening flowers in selected genotypes and observed that vegetative growth continued vigorously even after the start of flowering, consequently most flowers that opened early aborted, then new flowers initiated. Baba et al. (2003) reported more than two-thirds of the vegetative organs develop after flowering in soybean. A considerable reason for the abortion of early opening flowers may be the competition for assimilate supply between pod development and vegetative growth after flowering.

Compared with the days of R1–R3, variation in days of R3–R5 between early and late sowing did not show a positive correlation (Fig. 2-9). Khaliliaqdam (2014) reported that variation in the duration of R3–R5 with sowing time was limited and insignificant. Han et

al. (2006) reported that pod elongation (R3–R5) was delayed slightly by long day condition. From the above discussion, the duration of pod elongation (R3–R5) may not be responsive to photoperiod. However, the physiological basis of the response in this phase remains unclear. Additionally, days of R5−R7 showed significant relationship between early and late sowing (Fig. 2-10).

Present study pointed out that seed weight plant^{-1} was significantly related with DEF (Fig. 2-11 A), whereas insignificantly related with duration from first flower open to start of seed filling (Fig. 2-11 B). Similar result reported by Akhanda et al. (1981) and Nelson (1987). Moreover, several studies indicated that seed-filling period is positively correlated with seed weight (Hanway and Weber, 1971; Gay et al., 1980; Smith and Nelson, 1986). However, in our study seed-filling period showed insignificant correlations with the seed weight plant^{-1} (Fig. 2-11 C), which is contrast with previous reports. It is also found that seed weight plant^{-1} was positively correlated with whole growing period (Fig. 2-11 D), which is consistent with previous research (Fatichin et al., 2013).

Several previous studies identified the importance of node number in plant to yield formation (Board et al., 1990; Board and Tan, 1995; Board et al., 1997). In the present study, node number at flowering showed positive correlation with seed weight plant^{-1} (Fig. 2-12).

Some vegetative growth relating parameters (node number in main stem, total node number in plant, stem height, stem weight, and number of branches) showed significant and positive correlation with seed weight plant^{-1} (Fig. 2-13). Furthermore, vegetative growth increased with delayed flowering time. Therefore, delayed flowering genotypes

keep large vegetative growth, consequently produced high seed weight plant^{-1} in late sowing.

Soybean *GmWMC* genotypes exhibited a wide variation in seed weight plant^{-1} under short photoperiodic condition. More than two third genotypes showed less than 20 g plant^{-1} yield. However, some genotypes, i.e. Karasumame 'Naihou', Chiengmai Palmetto, Local Var. 'Tegineneng', Manshuu Masshok and Java 5 exhibited higher seed weight plant^{-1} (Table 2-1). These genotypes generally have longer DEF and large total node number. Large node number could be caused by the larger vegetative growth. As former genotypes give satisfactory seed weight plant^{-1} even under short photoperiodic condition (late sowing), these genotypes may adapt to tropical areas. Previous research has been reported genotypes of soybean such as Caviness, Parana, IAS-5, Akisengoku, and Akiyoshi showed higher productivity by late sowing in Saga, Japan. These genotypes generally have larger number of pod and seed filling periods but medium seed size (Fatichin et. al., 2013).

In conclusion, the results revealed that the duration of DEF, pod formation, and seed filling greatly declined by late sowing, but not on the pod elongation in *GmWMC* soybean. Additionally, late flowering genotypes produced large vegetative growth and higher seed weight plant^{-1} in soybean *GmWMC*, suggesting seed weight plant^{-1} of soybean could be enhanced by selecting genotypes in the late sowing based on longer DEF and vegetative growth. These results provided distinct information of the characteristics of *GmWMC* genotypes. However, the key problem with this research was that photoperiod and temperature changed daily and interact each other in the natural condition. Therefore, further experiment should be needed to find out the exact effect by either temperature or photoperiod in following chapters.

5. Summary

Short photoperiod could be causal factor for the low productivity of soybean in tropical areas. In order to increase soybean production in tropical areas, we tested the effect of photoperiod (sowing time) in different growth stages and investigated the yield potentiality by late sowing in the world soybean mini-core collections (*GmWMC*) including 82 genotypes provided by NARO gene bank of Japan. Seeds were sown in late May (early sowing) and early August (late sowing) from 2015 to 2017 in the field at Saga University, Japan. The daily average temperatures and photoperiodic hours were collected from weather stations. The average values of photoperiod and temperature were 15.13 h and 25.7°C in early sowing and 14.08 h and 28.2°C in late sowing from first emergence to the average date of flower open. The dates of emergence, first flowering (R1), first pod appearance (R3), the start of seed filling (R5), and beginning maturity (R7) were recorded. Additionally, seed weight plant^{-1} and some yield relating parameters at R8 stage were also recorded using five randomly selected plants under late sowing in 2015. The days from emergence to first flower open (DEF) were shortened by late sowing in all genotypes. The days of R1–R3 (for pod formation) and R5−R7 (seed filling) responded to the sowing time similarly to that for flowering. However, there were no such responses observed for days of R3–R5 (for pod elongation). Seed weight plant^{-1} varied from 1.4–39.5 g in *GmWMC* genotypes. More than 2/3 genotypes produced less seed weight plant^{-1} (< 20 g). Seed weight plant^{-1} was high and significantly correlated with DEF and days from emergence to R7 (whole growing period), whereas insignificantly correlated with the days from R1 – R5

(first flower open to start of seed filling) and days from R5 – R7 (seed filling). However, seed weight plant^{-1} showed positive correlation with node number in main stem, total node number in plant, stem height, stem weight, and number of branches per plant. Soybean genotypes, i. e. Karasumame 'Naihou' (39.53 g plant^{-1}), Chiengmai Palmetto (28.80 g plant^{-1}), Local Var. 'Tegineneng' (25.68 g plant^{-1}) and Manshuu Masshok (25.47 g plant^{-1}) produced higher seed weight plant^{-1}. These genotypes have sufficient vegetative growth such as larger total node number, longer stem height, and more branch number. These results indicated that delayed flowering and large vegetative growth increased seed yield in late sowing and result also provided distinct information of 82 *GmWMC* genotypes.

Chapter 3

The Impact of Temperature on Flowering in *GmWMC* Genotypes

1. Introduction

Soybean is a warm season crop and temperature is one of the most influential aspect to regulate soybean flowering. It is well known that the growth and development of soybean are affected by temperature (Hatfield and Prueger, 2015). Besides, temperature affect soybean seed yield. Duration between emergence and flowering has been known in Chapter 2 to be regulated by the interaction of photoperiod and temperature in the natural condition. It is reported that the involvement of a photoperiod and temperature interaction in the soybean flowering (Garner and Allard, 1930; Lawn and Byth, 1973). The problem could be outlined that determination of the effect of temperature in the natural condition is hard, since temperature and photoperiod regularly changed and interact each other.

In the field experiment (Chapter 2), the days from emergence to first flower open (DEF) was shortened by late sowing (short photoperiod and high temperature). However, the effects of photoperiod and temperature on soybean flowering were not possible to differentiate in the field experiments. In that case, the shortcoming of the results in Chapter 2 are the unclear causes by either photoperiod or temperature. In this Chapter the effect of temperature on soybean flowering has been extensively studied in the controlled environment to overcome this obstacle.

On the other hand, Constable and Rose (1988) used a multiple regression calculation technique to separate the effect of photoperiod and temperature from the natural condition and revealed that DEF was affected by photoperiod and temperature. Wu et al. (2015) calculated the independent and interactive photo-thermal effects on soybean flowering in the natural condition using a model, and reported that effect of photoperiod was greater under long-day than short-day condition as well as effect of temperature was greater under low temperature than high temperature. Both reports indicated that they used a model calculation to separate the photo-thermal effect, however in this study, an attempt was made to separate those effect.

Temperature has a positive influence on the rate of crop development. Liu et al. (2008) reported the favorable temperature for soybean flowering is 20–22°C. It has also been documented that high temperature stimulates flowering in soybean (Rahman et al., 2006; Kantolic and Slafer, 2007; Setiyono et al., 2007; Gaynor et al., 2011) and increases the rate of crop development (Craufurd and Wheeler, 2009). Roberts (1943) reported that temperature during the dark period is more important than temperature in the light period.

From this background, there is a major impact on the pattern of responses to temperature in soybean flowering. Chapter 2 revealed that DEF could be shortened by late sowing, which is caused by the interaction of short photoperiod and high temperature. Hence, it is necessary to make clear the independent effect by either photoperiod or temperature on soybean flowering in the natural condition. A series of growth chamber experiments were conducted to make clear the effect of temperature on flowering in soybean.

2. Materials and methods

Plant materials and growth conditions

Plants were grown in a growth chamber (KG-50 HLA, Koitotron Co., Ltd., Japan) (Fig. 3-1) in controlled temperatures and light system. In the first experiment, the photoperiod was set at 12 h, and the day/night temperatures were set as 25/18°C, 28/22°C, and 33/28°C for low, medium, and high temperature treatments, respectively. Cool white fluorescent and incandescent light (FPR96EX-D/A, Panasonic Co., Ltd., Japan) were used that produced 450 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density at about 1 m above the plants. Same plant materials with those in Chapter 2, eighty-two genotypes of the soybean world mini-core collections (*GmWMC*), were used. Five seeds were sown in the pots (15 cm diameter and 20 cm height) filled with sand and vermiculite (1:1 by volume) as the medium. Two plants were allowed to grow in each pot for each genotype, and the experiment were repeated two times. Standard nutrient solution which established in previous research (Zhao et al., 2014) in Table 3-1, was applied two times per week. Water was applied to prevent drying.

Table 3-1. Content of nutrient solution established by Zhao et al. (2014).

Nutrient	Concentration (ppm)	Reagents
N	50	NH_4NO_3
P	70	KH_2PO_4
K	110	K_2SO_4, KH_2PO_4
Mg	90	$MgSO_4$
Ca	35	$CaCl_2$
Fe	3.5	NaFeEDTA
Mn	0.3	$MnSo_4$
B	0.06	H_3BO_3
Zn	0.009	$ZnSO_4$
Cu	0.009	$CuSO_4$
Mo	0.009	MoO_3

Modified from Matsunaga et al. (1983).

The second experiment was conducted to determine the temperature effect widely from 20–34°C using 8 selected genotypes. The photoperiod was set at 10 h in order to accelerate flowering early. Two plants were allowed to grow in each pot for each genotype and the experiment was replicated three times.

Furthermore, the effect of fluctuated day/night temperature was also tested in the third experiment. Temperature were maintained at 26/26, 28/24, 30/22 and 34/18°C (day/night). Plant materials and replication were same as in second experiment.

Data collection

In all three experiments, the date of emergence (50% of plants with cotyledons above soil surface), first flowering (50% of plants with one flower at any node, R1) were determined according to Fehr et al. (1971).

Accumulated temperature and effective accumulated temperature

Accumulated temperature from emergence to first flower open (ATEF) was calculated. Effective accumulated temperature from emergence to first flower open (EATEF) for each genotype was determined by subtracting 10°C from the daily mean temperature and accumulated those from emergence to first flower open. The reason of subtracting 10°C is that soybean plant may not develop below this temperature.

Statistical analysis

Same correlations with significant level were measured like Chapter 2.

Fig. 3-1. The contents of used growth chamber.

3. Results

Effect of different temperature regime on soybean flowering

To determine the effect of temperature on flowering in soybean, first experiment was conducted with a 12 h photoperiod using three temperature conditions. The DEFs were 29–72 d at 25/18°C, 24–47 d at 28/22°C, and 19–40 d at 33/28°C, indicating clear shortening with increasing temperature (Fig. 3-2 A, D, and G). However, there were less-pronounced differences in ATEF (623–1548°C, 600–1187°C, and 579–1235°C at 25/18°C, 28/22°C, and 33/28°C, respectively) (Fig. 3-2 B, E, and H), and almost no apparent differences in EATEF (333–828°C, 360–712°C, and 389–830°C at 25/18°C, 28/22°C, and 33/28°C, respectively) (Fig. 3-2 C, F, and I) among three temperature conditions. Furthermore, the slopes of relationships between the three temperature conditions were closer to the line of 1:1 for ATEF and EATEF compared with DEF, indicating the ATEF or EATEF were less affected or unaffected by temperature in the most of the *GmWMC* genotypes.

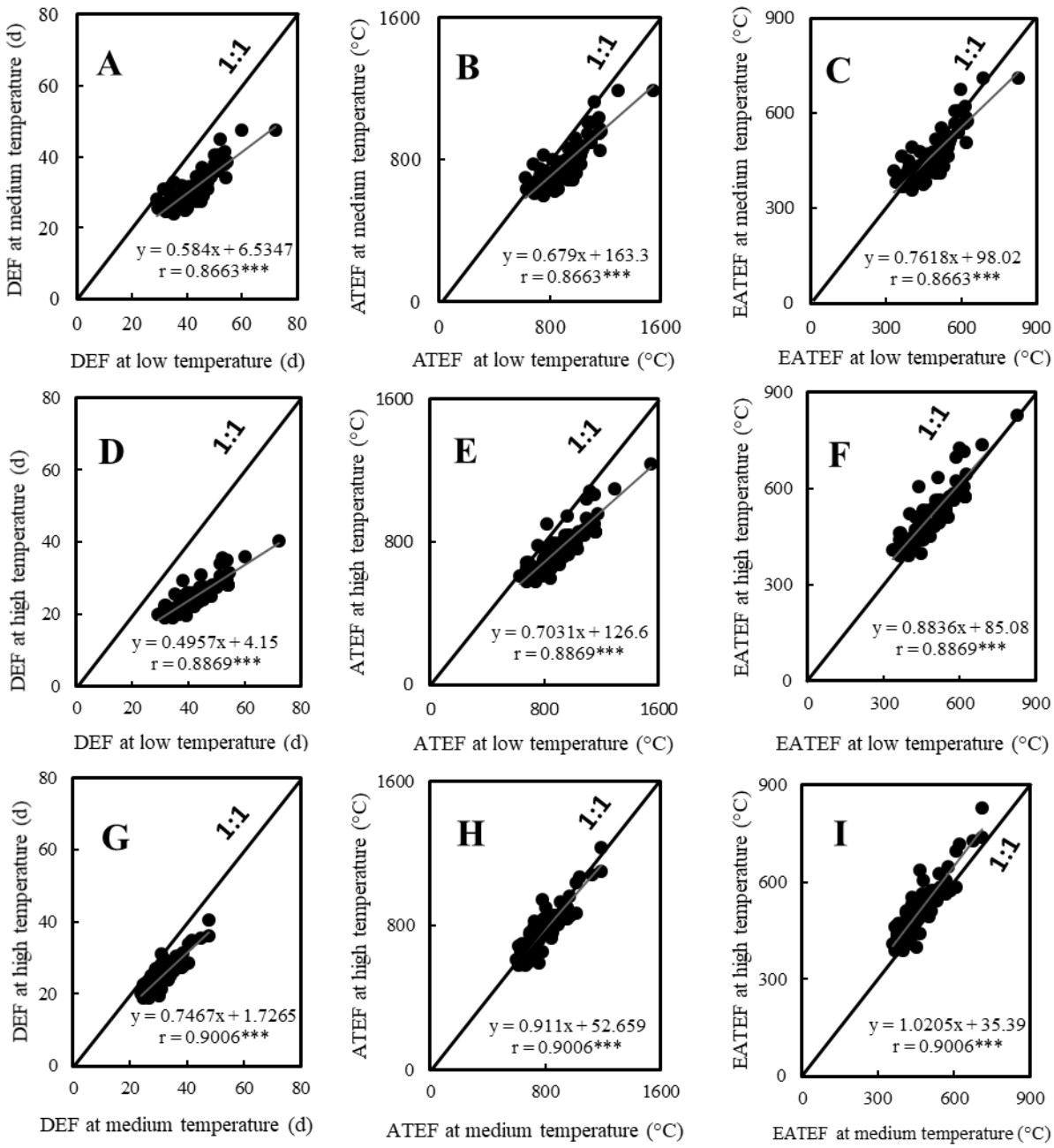

Fig. 3-2. Relationship among the days from emergence to first flower open (DEF), the accumulated temperatures during emergence to first flower open (ATEF) and the effective accumulated temperature from emergence to first flower open (EATEF) at low (25/18°C), medium (28/22°C) and high (33/28°C) day/night temperature. A, D, G: relationship for DEF; B, E, H: relationship for ATEF and C, F, I: relationship for EATEF. *** denotes significant at $P < 0.001$ and line represents the 1:1 ratio.

Additionally, some genotypes showed large ATEF (Sandek Sieng: 1118°C, 1175°C, and 1067°C; Miss 33 Dixi: 1548°C, 1150°C, and 1220°C at 25/18°C, 28/22°C, and 33/28°C, respectively) with a 12 h photoperiod (Fig. 3-2 B, E, and H), which could be caused by strong photosensitivity or a long juvenile growth phase, or both. Overall, the results of the growth chamber experiments showed that ATEF and EATEF varied widely between genotypes; however, they did not differ between the three temperature conditions within an individual genotype.

Effect of different constant temperature on soybean flowering

Fig. 3-3 shows the response of flowering to a range of temperatures from 20°C to 34°C with a 10 h photoperiod in eight selected genotypes. The DEF was shortened gradually by the increased temperature till 30°C, indicating higher temperature stimulates soybean flowering. However, DEF increased at more than 30°C in all genotypes.

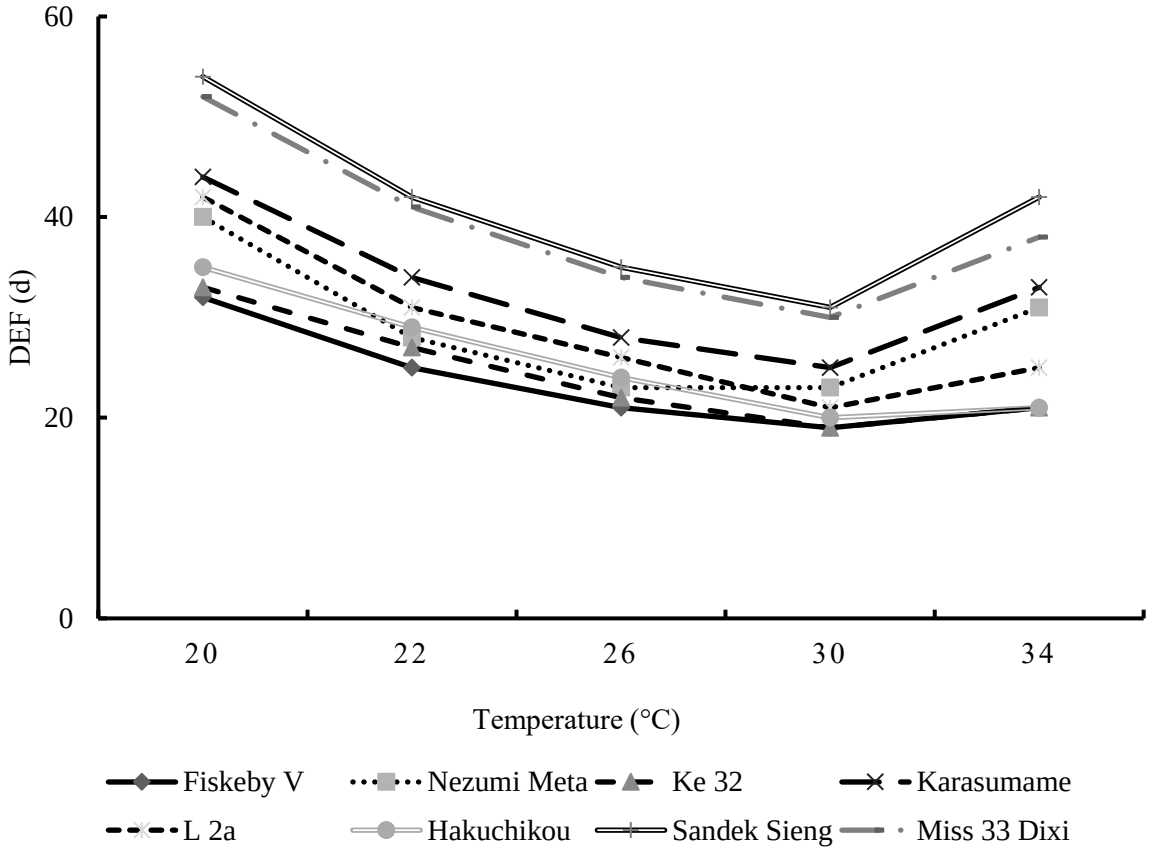

Fig. 3-3. Days from emergence to first flower open (DEF) at different temperatures under 10 h photoperiod in eight selected soybean genotypes.

On the other hand, ATEF of all genotypes were almost constant against the changes in temperature from 22 to 30°C (Fig.3-4), which is contrast that for DEF. This result was supported the previous result that ATEF is unaffected by temperature.

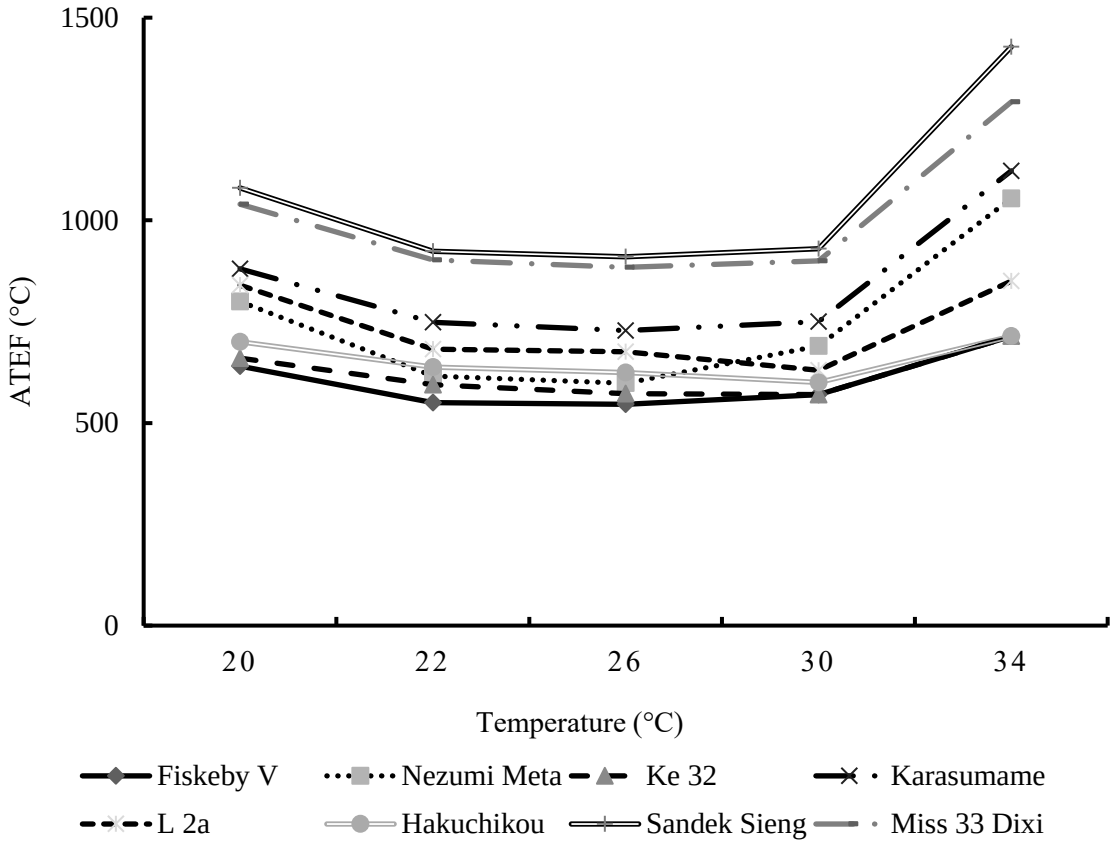

Fig. 3-4. Accumulated temperatures from emergence to first flower open (ATEF) at different temperatures under 10 h photoperiod in eight selected soybean genotypes.

In addition, temperatures of 20°C or 34°C caused higher ATEF values in all genotypes (Fig. 3-4), indicating slower rates of growth and development in these temperature conditions.

Effect of fluctuated day/night temperature on soybean flowering

In order to clarify the effect of fluctuated day/night temperature, response of flowering to three temperature regimes (26/26, 28/24, 30/22, 34/18°C, day/night) at 10 h photoperiod was exhibited in Fig. 3-5. The variation of ATEF in each genotype did not differ notably with the fluctuated day/night temperature, indicating soybean flowering was almost unaffected by fluctuated day/night temperature in the case that accumulated temperatures were same.

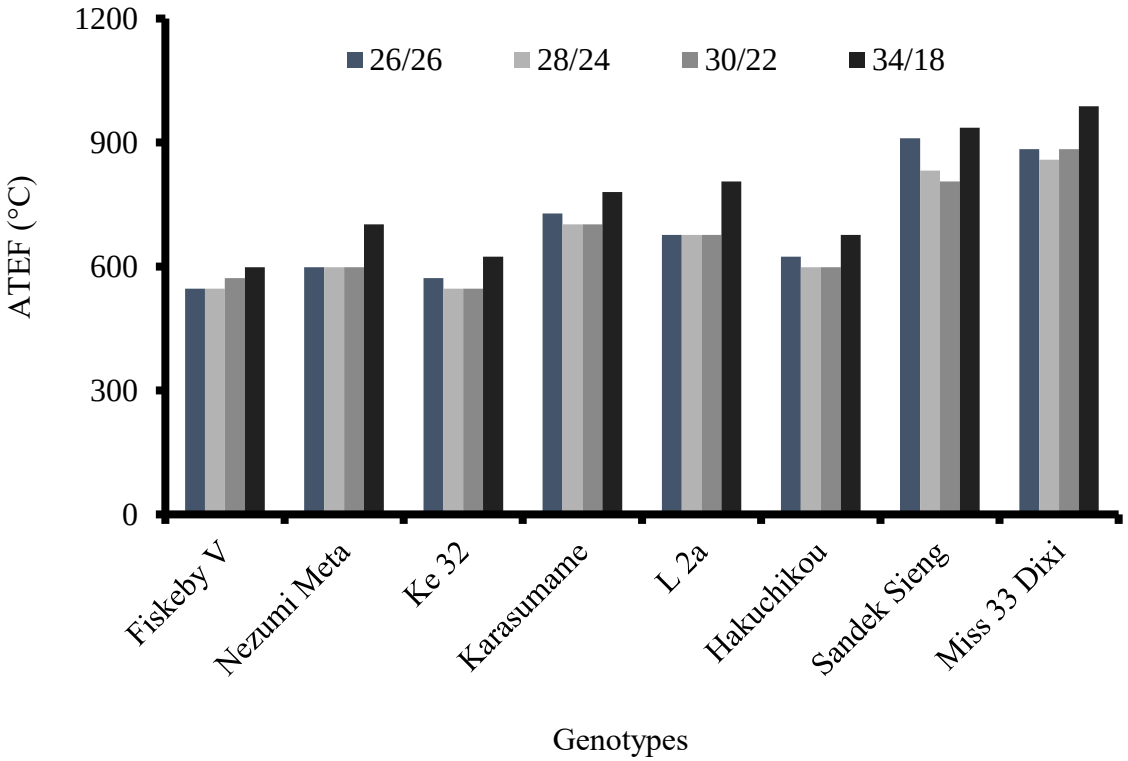

Fig. 3-5. Accumulated temperatures from emergence to first flower open (ATEF) under 26/26, 28/24, 30/22 and 34/18°C (day/night) temperature at 10 h photoperiod in eight selected genotypes.

Comparison between DEF and ATEF in the field experiment

Fig. 3-6 shows the relationship of DEF and ATEF between early and late sowing from 2015 to 2017. DEF was shorter in late sowing in all genotypes (Fig. 3-5 A, C, and E) whereas ATEF showed similar values in some early flowering genotypes (e.g. 596 and 596°C in Ke 32, 568 and 572°C in Fiskeby V, and 691 and 663°C in Choyoutou) between early and late sowing (Fig. 3-6 B, D, and F), indicating that these genotypes could be insensitive to photoperiod. Therefore, the differences in ATEF were smaller than those in DEF.

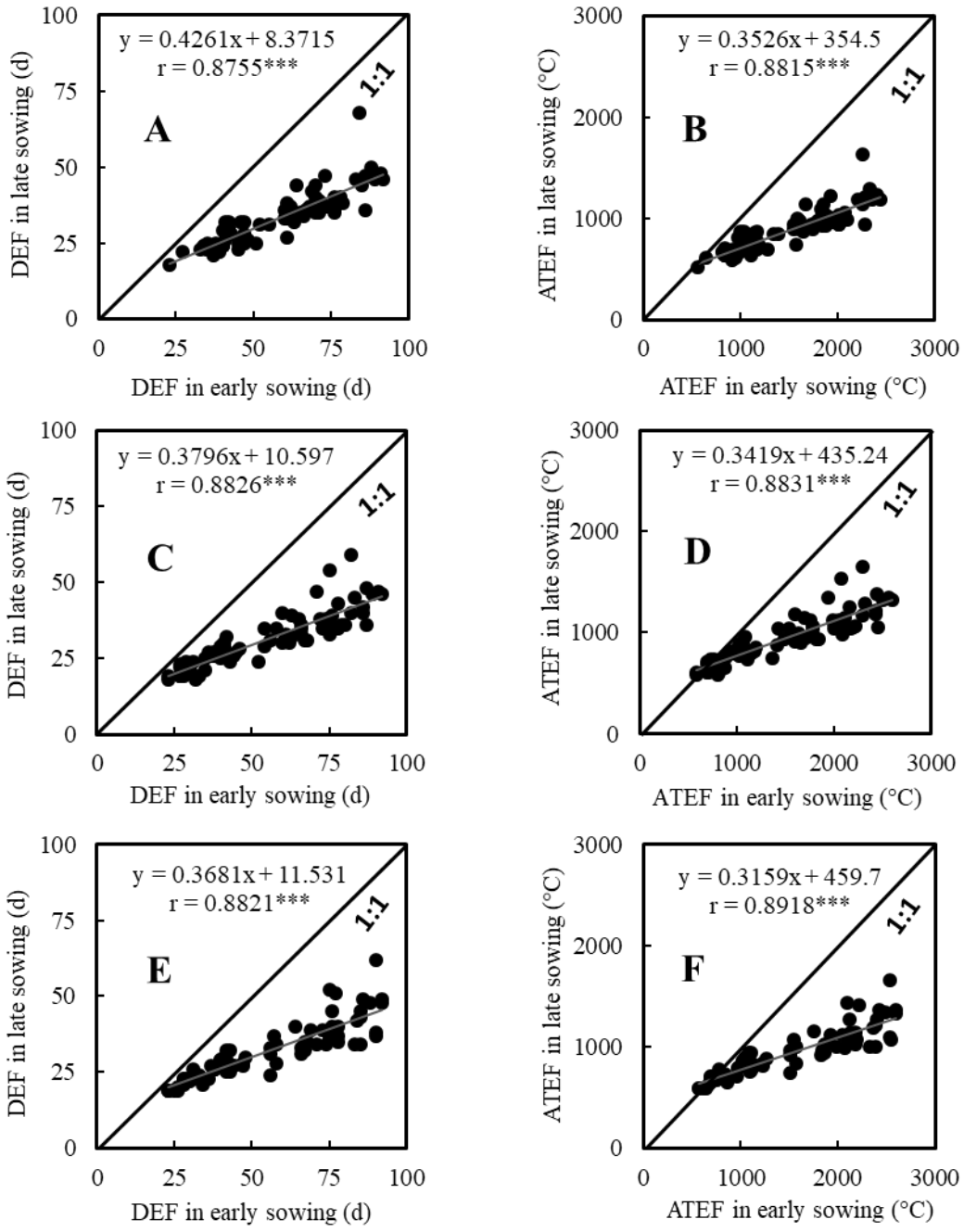

Fig. 3-6. Relationships of the days from emergence to first flower open (DEF) as well as the accumulated temperatures during emergence to first flower open (ATEF) between early and late sowing in 2015 (A, B), 2016 (C, D) and 2017 (E, F). *** denotes significant at $P < 0.001$ and line represents the 1:1 ratio.

4. Discussion

The field experiments in Chapter 2 indicated a problem that evaluation of the independent effect of photoperiod and temperature on soybean flowering was not feasible to separate in the natural conditions. This experiment was undertaken to evaluate the independent effect of temperature on soybean flowering.

Crop development of soybean basically depends on temperature. High temperature stimulates the growth rate of development, consequently decreasing the duration required to complete the developmental phase (Slafer and Rawson, 1994). It has been reported that higher temperatures alter plant phenology and accelerate flowering onset (Han et al., 2006). Our first experiment confirmed that high temperature shortened the DEF, but it had less effect on ATEF; however, there was almost no effect on EATEF regardless of the temperature conditions (Fig. 3-2). This implied that there were no differences in EATEF in most genotypes among the three temperature conditions tested. Thus, it could be considered that temperature affects soybean growth and development quantitatively; however, it may not have a triggering effect on flowering initiation. This indication was also illustrated in second experiment under a wide range of temperature (22 – 30°C) (Fig 3-4). Tacarindua et al. (2013) also reported that soybean flowering time could not be significantly affected by increasing temperature approximately 2–3°C from the natural temperature (25.9°C mean).

In addition, temperatures of 20°C or 34°C caused higher ATEF values in all genotypes (Fig. 3-4), indicating slower rates of growth and development in these temperature

conditions. Previous report also indicated that excessive low and high temperature reduced growth and development in soybean (Tacarindua et al., 2013). However, the main downside of the second experiment is that we used only constant temperature.

In the third experiment, day/night temperature of 26/26, 28/24, 30/22 and 34/18°C were imposed from emergence to flowering. Result obtained from this experiment that fluctuated day/night temperatures have almost no affect soybean flowering (Fig. 3-5). Ultimately, in the view of above three experiments, it can be confirmed that temperature affects soybean growth and development quantitatively and suggested that ATEF was preferable than DEF to evaluate temperature effect on flowering regardless of the conditions.

In the field experiment, the DEF values delayed by late sowing in all genotypes between 2015 and 2017 (Fig. 3-6 A, C, and E). The reduction in DEF in late sowing might be caused by both the shorter photoperiod and higher temperature. However, the ATEF values were the same in some early flowering genotypes between early and late sowing (Fig. 3-6 B, D, and F), indicating that the temperature effects could be minimized by using ATEF values in the field experiments. Major et al. (1975) observed that photoperiod was the dominant factor that affected flower opening in high temperature conditions. It has also been reported that early maturing genotypes responded more to temperature than photoperiod (Champman, 1986). Therefore, it could be stated that the use of ATEF could identify the effect of photoperiod independently from fluctuated temperature in natural conditions. Eventually, ATEF could be used instead of DEF to evaluate the sensitivity of soybean flowering to photoperiod in field experiments.

In addition, some genotypes, i.e. Sandek Sieng, Miss 33 Dixi, Hakuchikou, have higher ATEF with 12 h photoperiod in all temperature conditions (Fig. 3-6 B, D, and F), and these genotypes might maintain a long juvenile phase or strong photosensitivity even to 12 h photoperiod. Next Chapter may answer this question by an essential experiment.

Summary

In previous Chapter, there was a problem that photoperiod and temperature interact each other and changed seasonally at field experiment. Therefore, it was needed to identify separation effect by either photoperiod and temperature in soybean flowering. Our aim is to find out the effect of temperature on soybean flowering. As a result, in the first experiment we evaluated the variation in responses to temperature of soybean flowering in the world mini-core collections (*GmWMC*) including 82 genotypes provided by NARO gene bank of Japan. Seeds were sown in the growth chamber at Saga University, Japan. The temperatures were set as 25/18°C, 28/22°C, and 33/28°C (day/night) for low, medium, and high temperature treatments, respectively with 12 h photoperiod. The dates of emergence, first flowering (R1) were recorded. Effective accumulated temperature from emergence to first flower open (EATEF) for each genotype was calculated by subtracting 10°C from the daily mean temperature and accumulated those from emergence to first flower open. This experiment clearly demonstrated that the DEF was longer at lower temperature (25/18°C) than those at medium (28/22°C) and high (33/28°C) temperature; however, the ATEF and EATEF (effective ATEF) did not respond to the change in temperature. Moreover, some genotypes represented large ATEF (Sandek Sieng: 1118°C,

1175°C, and 1067°C; Miss 33 Dixi: 1548°C, 1150°C, and 1220°C at 25/18°C, 28/22°C, and 33/28°C, respectively) with a 12 h photoperiod, which could be resulted from strong photosensitivity or a long juvenile growth phase, or both. Second experiments confirmed that ATEF was not affected from 22 to 30°C temperatures. Afterward, for conformation the effect of fluctuated day/night temperature on soybean flowering we conducted another experiment using 26/26, 28/24, 30/22, 34/18°C, day/night temperature at 10 h photoperiod and result revealed that soybean flowering was unaffected with fluctuated day/night temperature. Above three experiments suggested that ATEF better than DEF to evaluate the effect of temperature on soybean flowering in field experiments. Finally, we evaluated the field data using accumulated temperature and result concluded that ATEF would be used to remove the effect of temperature on soybean flowering in field experiments. These results suggested that temperature might affect plant development quantitatively in soybean.

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Chapter 4

Variation of Photosensitivity and Juvenile Growth Phase in *GmWMC* Genotypes

1. Introduction

Soybean originated in temperate regions between 32° and 40° N latitude in China (Li et al., 2008). Nowadays, it is grown widely throughout tropical, subtropical, and temperate regions as one of the world's most important economic crops. A wide range of adaptation conditions were made easier mainly by the discovery of low photosensitive and long juvenile genotypes. The results in Chapter 3 demonstrated that some genotypes might maintain a long JGP or exhibit strong photosensitivity. However, conclusion was ambiguous whether genotypes show accurate JGP or strong photosensitivity.

It is generally accepted that soybean is a typical short-day crop. As a consequence, photosensitivity is a key factor for determining latitudinal adaption. It has also been recognized that photosensitivity of soybean genotypes controls the plant size, thereby affecting vegetative mass and yield potentiality (Shanmugasundaram and Tsou, 1978). Lu et al. (1967) reported that the variation in photosensitivity of soybean genotypes adapted to not only different location but also different season. Besides, photoperiod-insensitive genotypes could be grown regardless of photoperiod. Many researchers have introduced photoperiod-insensitive genotypes (Criswell and Hume, 1972; Huxley et al., 1974; Cregan and Hartwing, 1984).

Since Garner and Allard (1920) have found that the flowering in soybean plants respond to photoperiod, thereafter, there are a large number of studies on the photosensitivity of flowering in soybean, e.g., Criswell and Hume (1972) tested 111 soybean genotypes (maturity Group 00) with four photoperiods (12, 22, 23 and 24 h); Huxley et al. (1974) evaluated four soybean genotypes under 11:40 and 13:20 h photoperiod; Shanmugasundaram (1979) examined 40 genotypes under 16 h and 10 h photoperiod; Niwa (1985) tested seven soybean genotypes with four photoperiod (12, 12:40, 13:20 and 14 h). They all reported a wide variation between the genotypes, however, there was no specific method to evaluate photosensitivity standardly. Therefore, exact photosensitivity determination is very difficult for large number of genotypes, because the effective photoperiod differs among the genotypes.

Soybean cultivation in a large scale was difficult in tropical areas due to the lack of potential genotypes until the end of 1960s. Afterwards, this barrier was overcome with the identification of long JGP genotypes (Neumaier and James, 1993). These long JGP genotypes were first identified in Brazil and subsequently expanded the soybean production in regions of low latitude (Destro et al., 2001). Although 40% of soybean production areas are located below 24° S, Brazil has become the second largest soybean producer in the world and this has depended on the invention of long JGP genotypes (Spehar, 1995; Destro et al., 2001). Incorporation of the long JGP genotype into soybean germplasm adapted to one location may help the transfer of advantageous traits to genotypes adapted to another location. Moreover, JGP gives guidance to choose an

adaptable genotype for a specific latitude belt and supports soybean growers with more management adjustability in response to climatic conditions and crop rotation schemes. Since, the JGP is extremely important for the expansion of soybean production, particularly in tropical areas.

Most previous research focused mainly on genetic control of JGP. Ray et al. (1995) reported that long JGP is controlled by single recessive J/j locus. A later review described up to five genes that can control the JGP (Destro et al., 2001). To date, very little is known about JGP estimation in physiological way. Wilkerson et al. (1989) developed a method by moving plants from short- to long-photoperiod or the reverse to determine the JGP of soybean. However, this method is time consuming and laborious in cases where large number of genotypes are being studied. Therefore, it is necessary to establish a simple technique to evaluate the JGP in soybean.

On the other hand, in order to produce a genotype with certain vegetative growth that will be suited in tropical areas, either high photosensitivity or long JGP would be considered. However, the relations between photosensitivity and JGP are not well known.

Because both photosensitivity and juvenile growth could play a major role in expanding soybean adaption and enhance seed yield, the present study aimed to investigate the variation in photosensitivity and JGP in a wide range of genotypic backgrounds.

2. Materials and methods

Experimental design and growth conditions

A preliminary experiment was conducted to choose an effective photoperiod for evaluating photosensitivity in the growth chamber at Saga University, Japan. The control photoperiod was 8–14 h (2 h intervals) at 28/22°C (day/night) temperature. Two plants were allowed to grow in each pot for each genotype and replicated three times. The facilities and growth conditions throughout all the experiment in this Chapter were similar with in Chapter 3.

In the optimized conditions from a preliminary experiment, 82 genotypes of the World Mini-Core Collections (*GmWMC*) provided by the NARO gene bank of Japan (Table 4-1) were tested under long (13 h) and short (10 h) photoperiods at 28°C. We have chosen 28°C which is nearly similar condition in tropical areas, since our previous experiment (Chapter 3) concluded that temperature may do not have a triggering effect on flowering initiation but affect soybean growth and development quantitatively. The variations in photosensitivity and JGP were estimated in this experiment.

Data collection

Dates of emergence and first flowering were recorded like Chapter 3.

Index of photosensitivity of flowering (IPF)

The photosensitivity is measured in various ways depend on the aim of the experiment. In the present chapter, the photosensitivity of flowering in each genotype was calculated based on the difference in the accumulated temperature from emergence to first flower

open (ATEF) between long- (13 h) and short- (10 h) photoperiods at 28°C using the following equation:

$$IPF = 1 - ATEF_{10h} / ATEF_{13h}$$

where $ATEF_{10h}$ and $ATEF_{13h}$ are the accumulated temperatures from emergence to first flower open under short- and long- photoperiods. Here, we used ATEF instead of the days from emergence to first flower open (DEF) because our previous work (Chapter 3) proved that ATEF could represent photosensitivity better than DEF.

Estimation of relative JGP

DEF could be divided into three phases: A) pre-inductive phase, which is the JGP and insensitive to photoperiod; B) inductive phase, which is sensitive to photoperiod; and C) post-inductive phase, which is the duration for flower organs development and insensitive to photoperiod (Roberts and Summerfield, 1987; Ellis et al., 1992). We attempted to find out the most effective photoperiod (photoperiod which induction phase of most genotypes would be minimized) to get minimum DEF, and the variation in the minimum DEF was supposed to be caused by the JGP. Based on the above consideration, we evaluated that the differences in ATEF at the most effective photoperiodic conditions is the relative JGP (Fig. 4-1). Consequently, the relative JGP for each genotype was estimated by following equation:

Relative JGP = ATEF – Minimum ATEF (ATEF of the earliest flowering genotype).

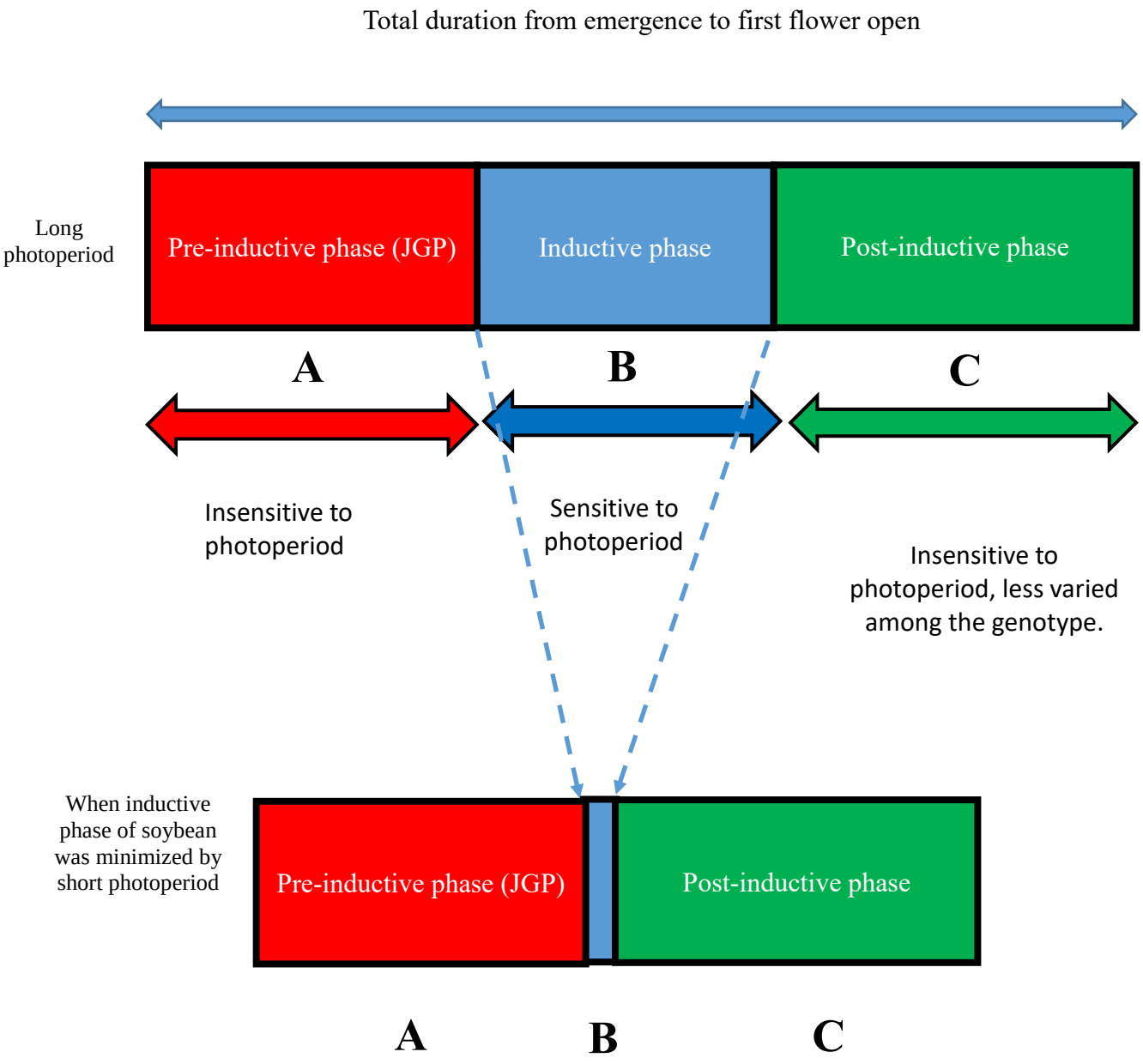

Fig. 4-1. Concept of juvenile growth phase (JGP) evaluation in soybean.

Statistical analysis

Single factor analysis of variance (ANOVA) and Tukey's HSD (honest significant difference) test were used for the analysis of significance ($p<0.05$) in eight selected genotypes among the different photoperiod in Table 4-1. Additionally, the correlations with significant level were measured at different figures.

3. Results

Flowering responses to a range of photoperiods

Table 4-1 shows the response of flowering to photoperiod from 8 to 14 h in selected 8 genotypes. The ATEF values were higher when the photoperiod was longer except for three insensitive genotypes (Fiskeby v, Ke 32, and Hakuchikou). However, the ATEF values were the lowest in 8 to 10 h photoperiod in all genotypes, implying that flowers opened earliest in these conditions. This result crucially suggested that an 8 or 10 h photoperiod could minimize the differences in the effect of photoperiod on flowering in all genotypes.

Table 4-1. Effect of different photoperiod on DEF at 28/22°C (day/night) temperature among eight selected soybean genotypes.

Photoperiod (h)	Fiskeby V	Nezumi Meta	Ke 32	Karasumame	L 2a	Hakuchikou	Sandek Sieng	Miss 33 Dixi
8	625 ± 0 b	625 ± 0 c	600 ± 0 ab	750 ± 0 d	625 ± 25 c	600 ± 0 ab	850 ± 25 b	875 ± 25 c
10	625 ± 0 b	650 ± 0 c	625 ± 0 a	825 ± 0 c	650 ± 0 c	625 ± 0 ab	875 ± 25 b	950 ± 0 b
12	675 ± 25 a	725 ± 25 b	625 ± 25 a	900 ± 0 b	750 ± 0 b	650 ± 25 a	1125 ± 25 a	1200 ± 0 a
14	675 ± 25 a	1425 ± 25 a	650 ± 25 a	950 ± 25 a	875 ± 25 a	650 ± 25 a	no flowering	no flowering

Data expressed as mean values ± SD of three replication. In a column, means followed by same letter are not significantly different at $p<0.05$ by Turkey Kramer test.

In addition, two genotypes (Sandek Sieng and Miss 33 Dixi) did not open flowers until 75 days after emergence at 14 h photoperiod (Table 4-1), indicating that these two genotypes might have a critical photoperiod lower than 14 h or it takes an extremely long time for the flowers to open.

Variation of photosensitivity

To evaluate photosensitivity comprehensively in all genotypes, the ATEF was tested under 10 and 13 h photoperiod at 28°C. Ten h was set for short photoperiod because the earliest flowering time was observed, as well as 13 h for long photoperiod because all genotypes opened the flower under this condition. ATEF varied notably from 560°C to 1372°C under 13 h photoperiod, whereas it was from 560°C to 868°C under 10 h photoperiod. Furthermore, the IPF differed greatly from 0.00–0.47 d at growth chamber condition (Table 4-2).

Table 4-2. ATEF, relative juvenile growth phase (JGP) and index of photosensitivity of flowering (IPF) in *GmWMC* genotypes.

ID number	Genotypes	Origin	$ATEF_{10\,h}$ (°C)	$ATEF_{13}$ (°C)	RJGP	IPF
*GmWMC*001	Fiskeby V	Sweden	588	588	28	0.00
*GmWMC*006	Ks 1034	Malaysia	560	588	0	0.05
*GmWMC*011	Seita	Rep.Korea	588	756	28	0.22
*GmWMC*012	Manshuu	China	602	728	42	0.17
*GmWMC*014	Kls 203	Rep. Korea	728	-	168	-
*GmWMC*015	Chuuhoku 2	Rep.Korea	560	644	0	0.13
*GmWMC*018	Rigai Seitou	China	602	896	42	0.33
*GmWMC*019	Chousenshu (Cha)	Korea	588	588	28	0.00
*GmWMC*020	Pochal	Taiwan	602	784	42	0.23
*GmWMC*022	Nezumi Meta	Korean Peninsula	574	952	14	0.40
*GmWMC*024	Chieneum Kong	Rep.Korea	560	672	0	0.17
*GmWMC*027	Kongnamul Kong	RepKorea	560	672	0	0.17
*GmWMC*029	Shirosota	korean Peninsula	602	644	42	0.07
*GmWMC*035	Pekin Dai Outou	China	588	588	28	0.00
*GmWMC*036	Masshokutou (Kou 502)	China	588	588	28	0.00
*GmWMC*038	Ichiguuhou	China	644	1064	84	0.39
*GmWMC*042	Masshokutou (Kou 503)	China	588	588	28	0.00
*GmWMC*045	Okjo	Rep.Korea	616	672	56	0.08
*GmWMC*046	Ke 32	Philippines	560	560	0	0.00
*GmWMC*048	Heamnam	Rep.Korea	616	896	56	0.31
*GmWMC*066	Heukdaelip	Rep.Korea	602	644	42	0.07
*GmWMC*070	Choyoutou	China	588	616	28	0.05
*GmWMC*071	Pk 73-54	India	588	784	28	0.25
*GmWMC*072	M 581	India	602	728	42	0.17
*GmWMC*073	Uronkon	Korean Peninsula	588	644	28	0.09
*GmWMC*075	Cheongye Myongtae	Rep.Korea	588	616	28	0.05
*GmWMC*083	Keumdu	Rep.Korea	588	672	28	0.13
*GmWMC*084	Peking	China	560	616	0	0.09
*GmWMC*086	Anto Shoukokutou	China	560	616	0	0.09
*GmWMC*089	Bongchunbaekjam	China	616	672	56	0.08
*GmWMC*094	Jeokgak	Rep.Korea	602	812	42	0.26
*GmWMC*103	Senyoutou	China	700	1120	140	0.38
*GmWMC*107	Hakka Zashi	China	560	672	0	0.17
*GmWMC*108	Karasumame	China	742	812	182	0.09
*GmWMC*113	Baritou 3 A	Indonesia	602	672	42	0.10
*GmWMC*115	Williams 82	USA	588	644	28	0.09
*GmWMC*118	Oudu	Rep.Korea	602	812	42	0.26
*GmWMC*119	Hakubi	China	672	672	112	0.00
*GmWMC*120	U 1416	Nepal	644	812	84	0.21
*GmWMC*122	Gapsanjaelae(I)	Rep. Korea	602	672	42	0.10
*GmWMC*123	N 2295	Nepal	560	868	0	0.35
*GmWMC*125	Bhatmas	Nepal	560	812	0	0.31
*GmWMC*129	Aoki Mame	China	630	-	70	-
*GmWMC*132	L 2a	Philippines	588	644	28	0.09
*GmWMC*136	Local Var (Seputih Raman)	Indonesia (Sumatra)	742	1148	182	0.35
*GmWMC*138	Col/Pak/1989/Ibpgr/2326(1)	Pakistan	560	728	0	0.23
*GmWMC*141	Petek	Indonesia	602	980	42	0.39
*GmWMC*142	Java 5	Indonesia	742	1260	182	0.41

ID number	Genotypes	Origin	$ATEF_{10\,h}$ (°C)	$ATEF_{13}$ (°C)	RJGP	IPF
*GmWMC*143	M 44	India	560	812	0	0.31
*GmWMC*144	M 918	India	616	1092	56	0.44
*GmWMC*146	Hm 39	India	602	868	42	0.31
*GmWMC*147	Col/Thai/1986/Thai-78	Thailand	602	812	42	0.26
*GmWMC*148	M 42	India	672	1260	112	0.47
*GmWMC*150	U 1042-1	Nepal	602	952	42	0.37
*GmWMC*151	Java 7	Indonesia	602	924	42	0.35
*GmWMC*152	U 1290-1	Nepal	616	1036	56	0.41
*GmWMC*154	Manshuu Masshokutou	China	616	1008	56	0.39
*GmWMC*156	U 8006-3	Nepal	644	1036	84	0.38
*GmWMC*159	Col/Pak/1989/Ibpgr/2323(2)	Pakistan	560	644	0	0.13
*GmWMC*160	N 2392	Nepal	644	-	84	-
*GmWMC*162	Col/Thai/1986/Thai-80	Thailand	616	924	56	0.33
*GmWMC*163	N 2491	Nepal	686	924	126	0.26
*GmWMC*165	Karasumame (Shinchiku)	Taiwan	700	700	140	0.00
*GmWMC*166	Merapi	Indonesia (Sumatra)	644	952	84	0.32
*GmWMC*168	L 317	India	672	1092	112	0.38
*GmWMC*169	Hakuchikou	China	560	616	0	0.09
*GmWMC*170	M 652	India	868	1176	308	0.26
*GmWMC*171	U-1741-2-2 No.3	Nepal	616	840	56	0.27
*GmWMC*173	Karasumame(Naihou)	Taiwan	812	1372	252	0.41
*GmWMC*175	Bishuu Daizu	China	602	812	42	0.26
*GmWMC*176	Sandek Sieng	Cambodia	840	1176	280	0.29
*GmWMC*181	Chiengmai Palmetto	Thailand	672	1036	112	0.35
*GmWMC*182	Local Var. (Tegineneng) Purple Flower	Indonesia (Sumatra)	742	1372	182	0.46
*GmWMC*182	Local Var. (Tegineneng) White Flower	Indonesia (Sumatra)	644	1120	84	0.43
*GmWMC*183	Karasumame (Heitou) Yellow Seed	Taiwan	644	728	84	0.12
*GmWMC*183	Karasumame (Heitou) Black Seed	Taiwan	714	784	154	0.09
*GmWMC*186	Ringgit	Indonesia (Sumatra)	630	1148	70	0.45
*GmWMC*187	Kadi Bhatto	Nepal	714	1288	154	0.45
*GmWMC*188	E C 112828	India	728	1316	168	0.45
*GmWMC*190	San Sai	Thailand	812	1148	252	0.29
*GmWMC*191	Miss 33 Dixi	Philippines	840	1344	280	0.38
*GmWMC*192	U 1155-4	Nepal	756	1092	196	0.31

$ATEF_{10h}$ is the accumulated temperatures from emergence to first flower open under 10 h photoperiod at 28°C temperature. $ATEF_{13h}$ is the accumulated temperatures from emergence to first flower open under 13 h photoperiod at 28°C temperature. IPF is the index of photosensitivity of flowering at growth chamber. Genotypes are arranged based on ID number. – denotes failed to collect data.

Variation of relative JGP

According to Table 4-1, the most effective photoperiod (photoperiod which induction phase of most genotypes were minimized) was found at 10 h because it produced the earliest flowering time. At this condition, the lowest ATEF (the ATEF of the earliest flowering genotypes) in *GmWMC* was 560°C (20 days at 28°C) in 14 genotypes. Therefore, the relative JGP for each genotype was evaluated by subtracting the critical lowest ATEF (560°C) from their ATEF value. Of note, the relative JGP varied from 0°C to 308°C in AT (0–11 days at 28°C) in *GmWMC* genotypes (Table 4-2). Furthermore, the frequency distribution pattern showed that the relative JGP did not vary so much and more than half of the genotypes were less than 60°C (not longer than two days) among the *GmWMC* (Fig. 4-2).

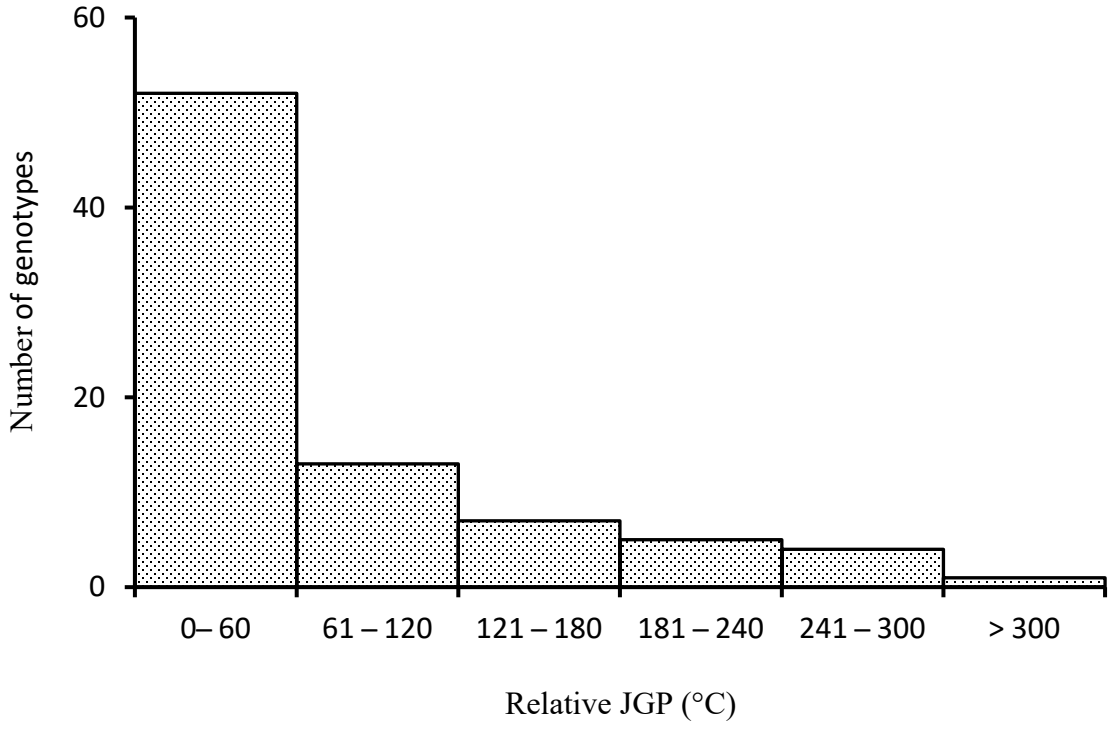

Fig. 4-2. Distribution of relative juvenile growth phase (JGP) among the *GmWMC* genotypes.

Relationship between IPF and relative JGP

There was a positive significant relationship between IPF at growth chamber and relative JGP ($r = 0.39$, $p < 0.001$) (Fig. 4-3); however, a wide distribution range was also observed in both IPF and relative JGP.

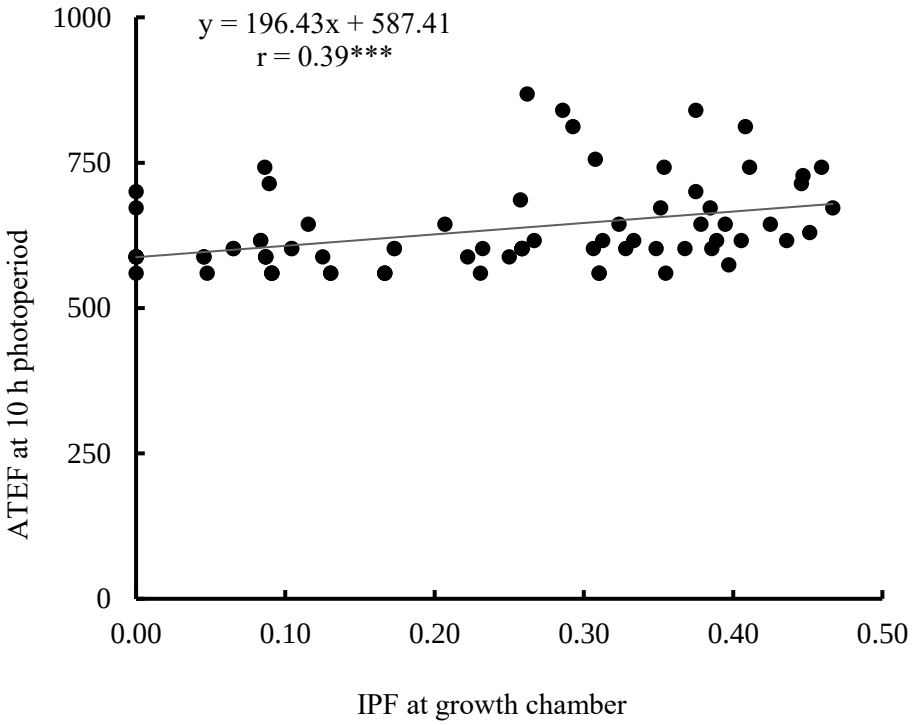

Fig. 4-3. Relationship between the accumulated temperatures from emergence to first flower open (ATEF) at 10 h photoperiod and index of photosensitivity of flowering (IPF) in the growth chamber. *** denotes significant at $P < 0.001$.

In addition, the most remarkable result from the data was that some genotypes exhibited special traits, namely low IPF with short relative JGP (Fiskeby V, Ke 32, Ks 1034), low IPF with medium relative JGP (Karasumame, Karasumame (Heitou) Black Seed), medium IPF with long relative JGP (M 652, Sandek Sieng), high IPF with short relative JGP (Nezumi Meta, M 918), and high IPF with long relative JGP (Miss 33 Dixi, Karasumame 'Naihou') (Table 4-3). These genotypes are originated from Sweden, Malaysia, Korea, China, Philippines, Taiwan, Thailand, Nepal, India, and Indonesia.

Table 4-3. Classification of *GmWMC* genotypes based on IPF and relative JGP.

ID number	Genotypes	Origin	IPF	$ATEF_{10h}$ (°C)	RJGP	Classification
*GmWMC*001	Fiskeby V	Sweden	0.00	588	28	low IPF with short RJGP
*GmWMC*006	Ks 1034	Malaysia	0.05	560	0	low IPF with short RJGP
*GmWMC*019	Chousenshu (Cha)	Korea	0.00	588	28	low IPF with short RJGP
*GmWMC*036	Masshokutou (Kou 502)	China	0.00	588	28	low IPF with short RJGP
*GmWMC*042	Masshokutou (Kou 503)	China	0.00	588	28	low IPF with short RJGP
*GmWMC*046	Ke 32	Philippines	0.00	560	0	low IPF with short RJGP
*GmWMC*108	Karasumame	China	0.09	742	182	low IPF with medium RJGP
*GmWMC*165	Karasumame(Shinchiku)	Taiwan	0.00	700	140	low IPF with medium RJGP
*GmWMC*183	Karasumame (Heitou) Black Seed	Taiwan	0.09	714	154	low IPF with medium RJGP
*GmWMC*170	M 652	India	0.26	868	308	medium IPF with long RJGP
*GmWMC*176	Sandek Sieng	Cambodia	0.29	840	280	medium IPF with long RJGP
*GmWMC*190	San Sai	Thailand	0.29	812	252	medium IPF with long RJGP
*GmWMC*192	U 1155-4	Nepal	0.31	756	196	medium IPF with long RJGP
*GmWMC*022	Nezumi Meta	Korean Peninsula	0.40	574	14	high IPF with short RJGP
*GmWMC*144	M 918	India	0.44	616	56	high IPF with short RJGP
*GmWMC*152	U 1290-1	Nepal	0.41	616	56	high IPF with short RJGP
*GmWMC*182	Local Var. (Tegineneng) Purple Flower	Indonesia (Sumatra)	0.46	742	182	high IPF with long RJGP
*GmWMC*173	Karasumame(Naihou)	Taiwan	0.41	812	252	high IPF with long RJGP
*GmWMC*191	Miss 33 Dixi	Philippines	0.38	840	280	high IPF with long RJGP

IPF is the index of photosensitivity of flowering at growth chamber. $ATEF_{10h}$ is the accumulated temperatures from emergence to first flower open under 10 h photoperiod at 28°C temperature. RJGP is the relative juvenile growth phase.

4. Discussion

Both photosensitivity and JGP are major factors that regulate latitudinal adaption of soybean. Optimal growth, development and seed yield are obtained when soybean is grown in its region of optimum adaptation. Long JGP genotypes facilitates the adaptability in tropical areas. The previous Chapter exhibited some long JGP or strong photosensitive genotypes, however there was a dilemma that whether those genotypes exactly maintain long JGP or strong photosensitivity. In this Chapter, the variation of photosensitivity and JGP were tested in all *GmWMC* genotypes.

Eight genotypes exhibited distinct variation of ATEF by different photoperiod responses. Furthermore, data also showed the shortest ATEF between 8 and 10 h photoperiod in all genotypes (Table 4-1), indicating 8 – 10 h were the photoperiod to get the earliest flowering. Cober (2011) tested four genotypes i.e. Parana, Paranagoiana, PI 159925, and X5063-39 with 3, 4, 5, 6, 8, and 12 h photoperiod at 25°C (day/night) temperature and reported that days from emergence to flowering were the shortest at 6 to 8 h photoperiod in all genotypes.

In addition, two genotypes (Sandek Sieng from Cambodia and Miss 33 Dixi from Philipines) did not initiate flowers until 75 days after emergence under 14 h photoperiod (Table 4-1). It could be considered that these two genotypes might have critical photoperiod lower than 14 h or take long time to initiate flower.

The range of IPF varied from 0.00 to 0.47 at growth chamber, and this variation was not affiliated with the origin of the genotypes (Table 4-2). This genotypic diversity may be associated with their genetic backgrounds and could be useful for crop improvement as well as for efficient management and conservation of germplasm resources. With the same

conception, the IPF at field condition was also calculated based on differences in ATEF between early sowing (long photoperiod) and late sowing (short photoperiod). Although strongly positive correlation ($r = 0.81$, $p<0.001$) was found in IPF between field and growth chamber, there were some variations, such as Pekin Dai Outou, Okjo and Ringgit genotypes showed 0.00, 0.08 and 0.45 in IPF at growth chamber, whereas showed 0.24, 0.34 and 0.36 at field, respectively (Table 4-2 and Fig. 4-4). Variation in the same genotype between field and growth chamber could be caused by different photoperiod. Additionally, result (- 0.01 to 0.58) was almost same and well responded to the results in growth chamber (Fig. 4-4). However, the key problem with the field experimental research was that photoperiod and temperature changed daily. The range of photosensitivity for each genotype at growth chamber showed little discrepancies compared with field study.

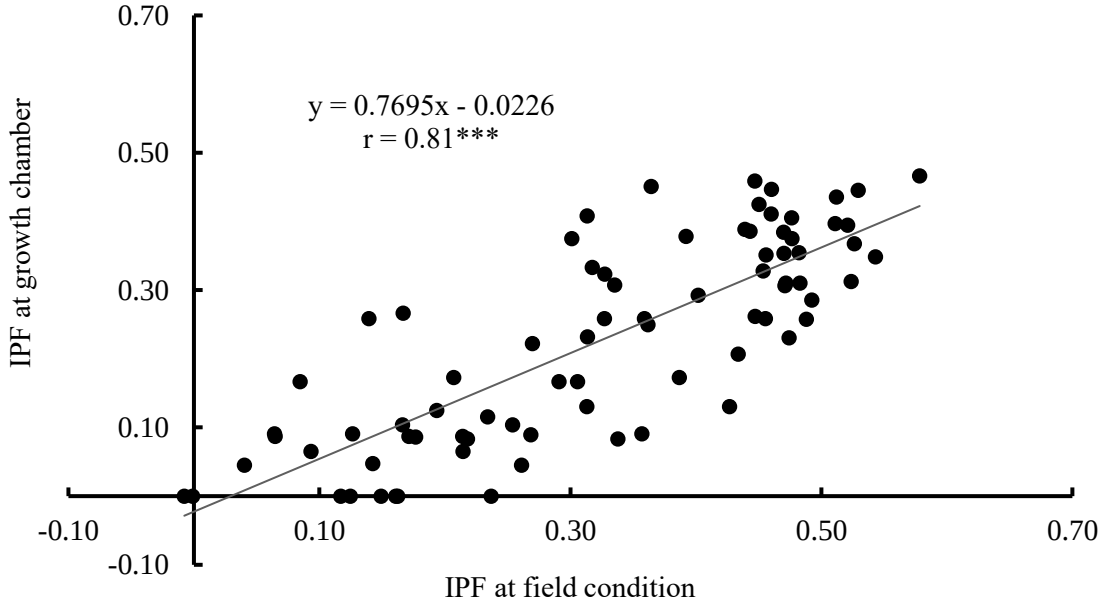

Fig. 4-4. Relationship between index of photosensitivity of flowering (IPF) in growth chamber and field conditions.

Furthermore, our results at growth chamber also showed that there are 8 genotypes with very low photosensitivity (IPF=0.00), and 10 genotypes with high photosensitivity (IPF>0.40) (Table 4-2). The former genotypes are originated mainly from Sweden, Korea, China and Philippines, and later from India, Indonesia, and Nepal. These high genotypic diversities would be potential source to expand the genetic base of soybean for wide adaptation areas, particularly tropical areas.

JGP is the limiting factor for soybean seed yield in tropical areas. It is hard to determine exact JGP because of the difficulty to separate the effects on emergence to flowering by individual phase. However, Wilkerson et al. (1989) developed a method by transferring plants from short to long photoperiod or the reverse to determine the JGP of soybean. This method had limitations related to photoperiod selection. For instance, Davis soybean was transferred from long (22 h) to short (9 h) photoperiod and vice versa at a constant temperature of 26°C, it showed 4 d of JGP (Wilkerson et al., 1989). When it was transferred from (16–18 h) to 12 h photoperiod and vice versa at a mean temperature of 25°C, it exhibited an 18 d of JGP (Ellis et al., 1992). The variation in JGP (4 d vs 18 d) could be observed for the same soybean genotype, caused by different photoperiod selection (9 h vs 12 h). Collinson et al. (1993) used a similar method and transferred four genotypes from a 11.5-h to 13.5-h photoperiod and vice versa at a mean temperature of 25°C and identified that JGP varied from 11 d to 33 d among four genotypes. Wang et al. (1998) used Hutcheson soybean and transferred from a 22-h photoperiod to 8-, 10-, 12-, or 14-h photoperiods at a constant temperature of 26°C and concluded that there was no JGP in Hutcheson soybean. Cober (2011) worked with four genotypes using a wide variation in photoperiod (2–16 h) at a constant temperature of 25°C and reported that Paranagoiana

soybean may have a 5 d JGP by comparing genotypes based on photoperiod response. All these reports indicated that there is a wide variability in JGP, which causes complexity with estimation processes, especially for appropriate photoperiod selection. Therefore, the considerable differences among reports could be caused by different estimation methods. Consequently, we tried to provide an easy technique for JGP evaluation. When the inductive phase is shortened in maximum, DEF is consisted of pre-inductive and post-inductive phases, however post inductive phase is considered less varied among the genotypes. Therefore, the DEF under short photoperiod in this experiment could be resulted mostly by JGP. The estimated relative JGP varied from 0 d to 11 d from emergence at 28°C in 82 *GmWMC* genotypes (Table 4-2). Indeed, the existing variability indicated the presence of genotypic differences in JGP. Several reports have also stated that the duration of the JGP could be varied by genotypes. (Shanmugasundaram and Tsou, 1978; Board and Settimi, 1988; Wilkerson et al., 1989; Upadhyay et al., 1994). Considering the evidence that there is no JGP in some genotypes, we predicted that the earliest flowering genotypes with 560°C AT in 82 *GmWMC* genotypes might not have a JGP. As a result, the ATEF of the earliest flowering genotypes (560°C or 20 days at 28°C) could be considered as a post- inductive period. Saitoh et al. (1999) also reported that this phase is around 20 days. Therefore, compared with others, our experiment may be easier to conduct when examining large numbers of genotypes.

Moreover, even there was a positive relationship between IPF and relative JGP, but this relative JGP was not always associated with IPS (Fig. 4-3). It implies that relative JGP may be partially independent of photosensitivity in soybean. Additionally, data provided important insight into the introduction of several special genotypes, namely low IPF with

short relative JGP (Fiskeby V, Ke 32, Ks 1034), low IPF with medium relative JGP (Karasumame, Karasumame (Heitou) Black Seed), medium IPF with long relative JGP (M 652, Sandek Sieng), high IPF with short relative JGP (Nezumi Meta, M 918), and high IPF with long relative JGP (Miss 33 Dixi, Karasumame 'Naihou') (Table 4-3). These genotypes may have unique genetic backgrounds and be useful in the development of new genotypes. Furthermore, as above mentioned, some long relative JGP genotypes may be good resources to broaden the adaption in tropical areas by ensuring sufficient vegetative growth even in short photoperiod condition.

5. Summary

Photosensitivity and juvenile growth phase (JGP) are the main yield limiting factors for soybean production in tropical areas. Previous Chapter showed that some genotypes might maintain a long JGP or strong photosensitivity even to 12 h photoperiod. Objective of this Chapter was to evaluate the variation in photosensitivity and JGP in the World Soybean Mini-Core Collections (*GmWMC*) including 82 genotypes. A preliminary experiment was conducted to choose an effective photoperiod for evaluating photosensitivity in the growth chamber. The control photoperiod was 8–14 h (2 h intervals) at 28/22°C (day/night) temperature. Eight soybean genotypes were used according to previous Chapter by considering the variation of photoperiod sensitivity. Emergence (50% of plants with cotyledons above soil surface), first flower initiation date (50% of plants with one flower at any node, R1) were recorded. The earliest flowering was observed at 8-10 h photoperiod in the preliminary experiment. Afterwards, we tested *GmWMC* genotypes under long (13 h) and short (10 h) photoperiods at 28°C temperature. Because 10 h was the photoperiod which induction phase of most genotypes were minimized, and all genotypes opened the flower under 13 h photoperiod. Index of photosensitivity of flowering (IPF) in each genotype was calculated based on the following equation: $1-ATEF_{10h} / ATEF_{13h}$, where $ATEF_{10h}$ and $ATEF_{13h}$ are the accumulated temperatures from emergence to flowering under short (10 h) and long photoperiods (13 h), respectively. Result showed that the IPF were 0.00 to 0.47 in *GmWMC* genotypes. Since DEF was minimum under 10 h photoperiod in most of the genotypes, the variation in the minimum DEF under this photoperiod was supposed to be caused by the relative JGP. Based on this consideration, we estimated the differences in ATEF under 10 h photoperiod, condition is the relative JGP. Relative JGP

varied from 0 to 308°C in accumulated temperature (0–11 days at 28°C) in *GmWMC* genotypes. Additionally, there was a positive relationship between IPF and JGP. However, a wide distribution range was also found in both IPF and relative JGP, indicating JGP may be partially independent of IPF in soybean. The data also introduced some special traits in some genotypes including low IPF with medium relative JGP (Karasumame, Karasumame (Heitou) Black Seed) and medium IPF with long relative JGP (M 652, Sandek Sieng). Results provided the information of the existence of genotypes with various combinations of IPF and JGP. Existence genotypes were the notable accomplishment for future breeding program to raise seed yield in tropical areas.

Chapter 5

General Discussion

Soybean is the primary source of vegetable proteins in the world today for human health, such as improve metabolism, help in protect heart disease, and defend against prostate and breast cancer. Besides, it is an extremely important source of protein feed supplement for livestock and used as a good source of environment friendly biodiesel (Clemente and Cahoon, 2009). The soybean production in most of the tropical Asian countries (Indonesia, Japan, China, Taiwan, Korea, Thailand, and Vietnam) are very few compared to other countries such as United states, Brazil and Argentina.

The main reason of low production in tropical areas is poor seed yield, caused by insufficient vegetative growth. Insufficient vegetative growth is led by early flowering. Early flowering is resulted from short photoperiod (Board and Settimi, 1986) or the joint effect of short photoperiod and high temperature (Board and Hall, 1984). However, the demand of soybean is high in tropical Asian countries, consequently they import soybean mostly from the United states and Brazil (Fig. 5-1).

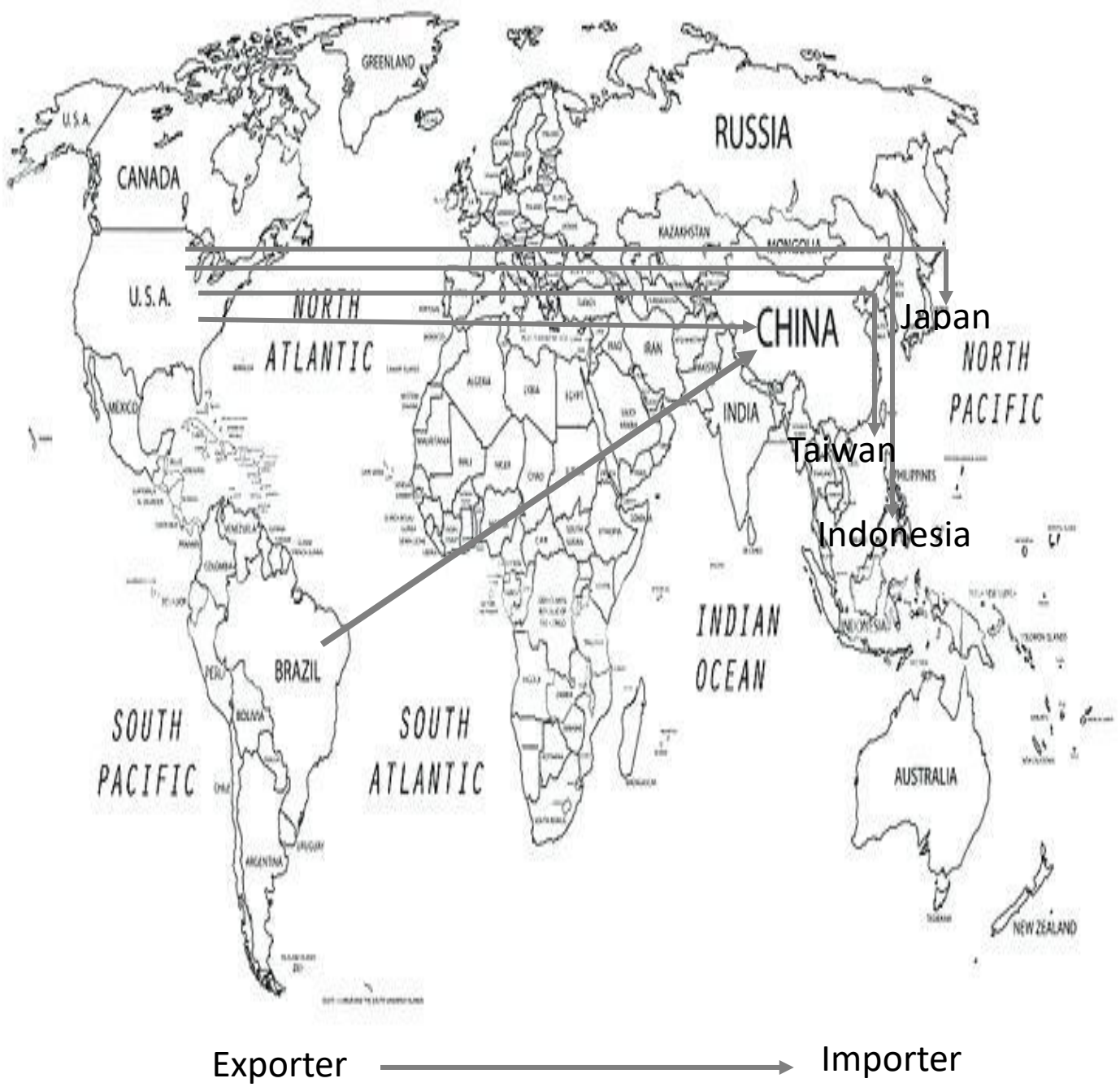

Fig. 5-1. Top Asian soybean importer countries.

To increase soybean production in tropical areas, it is necessary to introduce some genotypes with sufficient vegetative growth. In order to obtain genotypes including sufficient vegetative growth, the sensitivities to photoperiod and temperature on soybean flowering as well as juvenile growth phase (JGP) could be a desirable topic. The primary aim of this study was to provide profound knowledge about the effect of photoperiod and temperature on different growth stages of soybean; and then by using this knowledge to address a simple technique for JGP evaluation, ultimately, to provide important information to increase production potentiality for soybean in tropical areas.

Both pre- and post- flowering phases are responsible for soybean seed yield. Guthrie (1972) reported that all the stages of development in soybeans responded to photoperiod. Han and Wang (1995) reported that the effect of photoperiod was found not only on the duration of flowering and podding but also on the seed filling stages. Chapter 2 provided an overview about the effect of photoperiod (sowing time) on different growth stages and other yield components of soybean using worldwide soybean genotypes. The experiments demonstrated that duration of the stage of flowering, pod formation, and seed filling shortened by short photoperiod, however duration of pod elongation did not respond to photoperiod in *GmWMC* genotypes. Moreover, delayed flowering genotypes gave large vegetative mass and higher seed yield in *GmWMC* genotypes. Considering the overall results in Chapter 2, it could be suggested that seed yield would be enhanced to tropical areas by screening genotypes under short photoperiod according to delayed flowering time and large vegetative mass.

In Chapter 3, first experiment concluded that high temperature shortened the DEF, however temperature had almost no effect on ATEF or EATEF in case of three different temperature conditions, indicating ATEF could represent photosensitivity better than DEF in the natural condition. Further experiments illustrated that ATEF was not affected at 22 – 30°C regardless of either same or different day/night temperatures. Finally, it is indicated that ATEF could separate the effect of photoperiod independently from the effect of temperature in natural conditions. According to these results, it could be concluded that temperature affects soybean growth and development quantitatively. Han et al. (2006) reported that higher temperatures stimulate flowering onset in soybean. However, our result indicated that temperature may not have a triggering effect on flowering initiation in soybean. This conclusion differs from Han et al. (2006).

Both photosensitivity and JGP are the yield limiting factors for soybean production in tropical areas, therefore knowledge about the photosensitivity and JGP would help to enhance soybean seed yield to those areas. Therefore, in Chapter 4 we evaluated the index of photosensitivity (IPF) and relative JGP of *GmWMC* genotypes. Result showed that IPF varied from 0.00 to 0.47 and relative JGP varied from 0 to 308°C in accumulated temperature (0–11 days at 28°C) in *GmWMC* genotypes. Besides, seed weight plant^{-1} showed positive correlation with both IPF ($r = 0.57$, $p < 0.001$) and relative JGP ($r = 0.38$, $p < 0.001$), however IPF may be more effective to increase seed yield in tropical areas because of high correlation compared with relative JGP (Fig. 5-2).

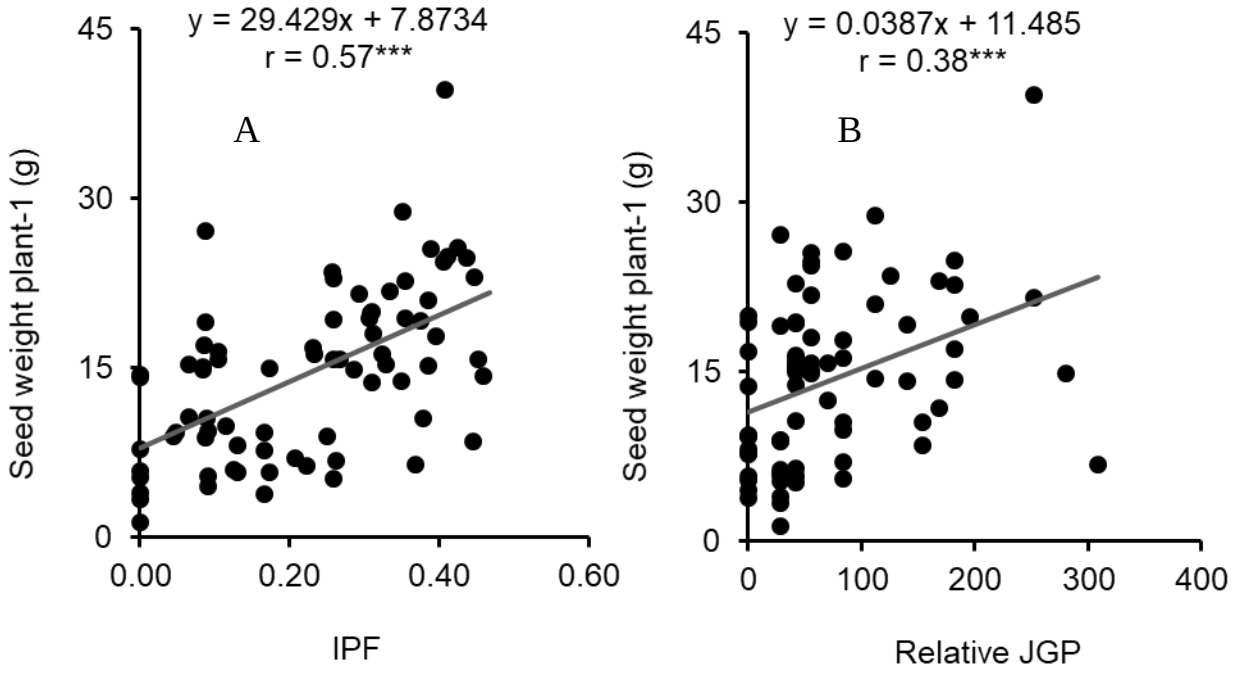

Fig. 5-2. Relationship of seed weight plant^{-1} with IPF (A) and relative JGP (B). *** denotes significant at $P < 0.001$.

A significant positive relationship was found between IPF and relative JGP ($r = 0.39$, $p < 0.001$), however wide distribution range was also observed (Fig. 5-3). This result indicated JGP may be partially independent from photosensitivity in soybean. Data also provided important insights into the introduction of several special genotypes, i.e. higher IPF but shorter relative JGP with high seed yield (Chiengmal palmetto, M918), or higher IPF and longer relative JGP with high seed yield (Karasumame 'Naihou', EC 112828). These special genotypes may be good resources to broaden the adaption in tropical areas by ensuring sufficient vegetative growth even under short photoperiodic condition.

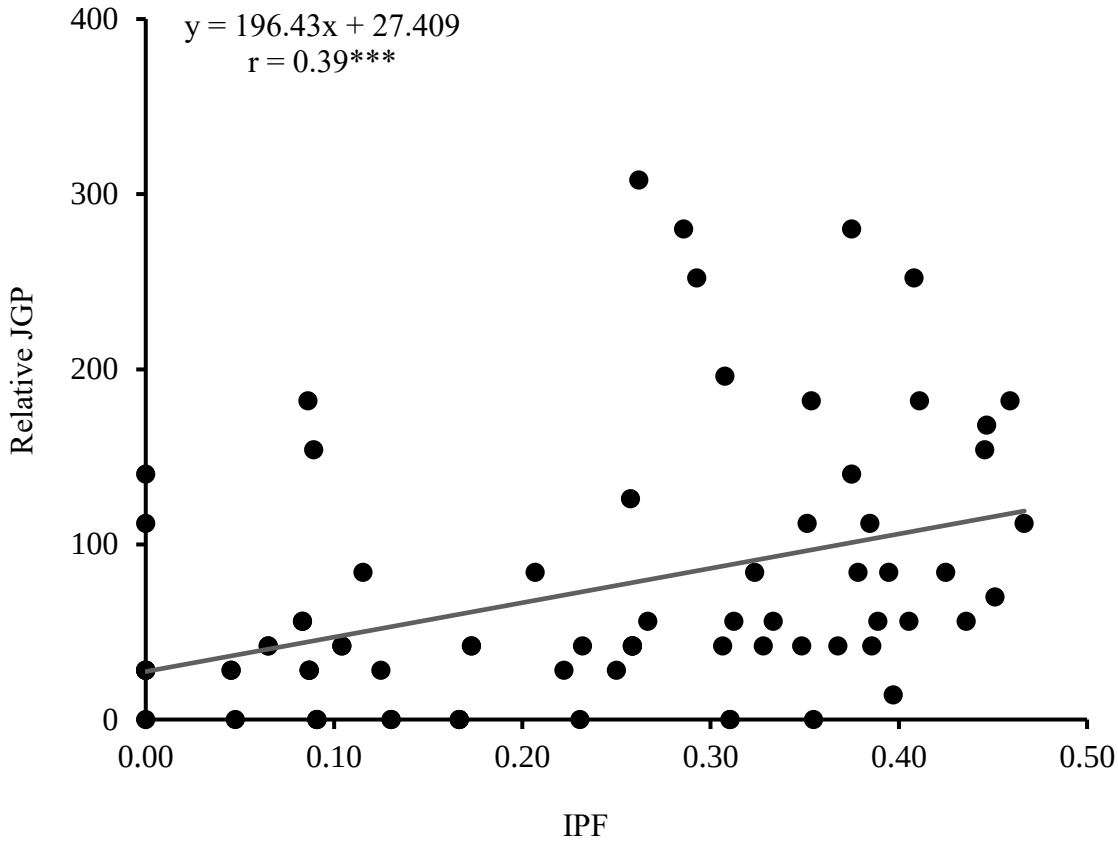

Fig. 5-3. Relationship between relative JGP and IPF *** denotes significant at $P < 0.001$.

Our main aim of this research was to extend soybean production in tropical areas. Miranda et al. (1990) conducted a breeding work using Parana, Davis, Hardee, Hill, and Santa Rosa genotypes including genes for long JGP in São Paulo State, Brazil (23°31' S), and released a genotype (IAC-15) that produced high plant height and seed yield. Hence, future work will be focused on soybean breeding programs using special genotypes that may help to extend soybean production in tropical areas.

Summary

The demand for soybeans in tropical Asia as a major protein resource has been increasing. However, the proportion of self-supply in most tropical Asian countries are extremely low because of low productivity. Soybean production in those areas face many problems, including short photoperiod and high temperature. Short photoperiod and high temperature could stimulate flower open early, result in insufficient vegetative growth therefore low seed yield. To assure certain vegetative growth, the sensitivities to photoperiod and temperature, and the juvenile growth phase (JGP) property could be key factors to determine desirable genotypes for those areas. In order to determine a suitable soybean genotype adaptable to tropical area, a series of experiments were conducted for the evaluation of variation in photo- and thermo- sensitivities using 82 genotypes of soybean world mini-core collections (*GmWMC*).

At field condition, *GmWMC* genotypes were sown in late May (for long photoperiod) and early August (for short photoperiod). The days from emergence to first flower open (DEF) ranged 23-92 days under long photoperiod, but 19-63 days under short photoperiod. The DEF were shortened by short photoperiod in all genotypes. Same trends were also observed in the duration for pod formation and seed filling, but not for pod elongation. Seed weight plant^{-1} under short photoperiod varied widely (1.4−39.5 g), it was highly correlated with DEF ($r = 0.61$, $p<0.001$), stem height ($r = 0.55$, $p<0.001$), number of branch ($r = 0.59$, $p<0.001$), and total node number ($r = 0.66$, $p<0.001$), indicating low seed weight plant^{-1} was clearly caused by less vegetative growth. Besides, data also introduced some high yielding genotypes, i.e. Karasumame 'Naihou' (39.5 g plant^{-1}), Chiengmai Palmetto

(28.80 g plant^{-1}), and Local Var. 'Tegineneng' (25.68 g plant^{-1}). Results indicated that late flowering genotypes with enough vegetative mass could be used as a selection index for improving soybean yield potential in tropical areas.

Because above mentioned responses under field condition are the results of interaction of fluctuated photoperiod and temperature seasonally, to clarify the effect of temperature independently on soybean flowering, we conducted three experiments in the controlled environment. The first experiment was conducted under 25/18°C, 28/22°C, and 33/28°C day/night temperatures. Results showed that DEF was longer in lower temperature in all genotypes; however, the accumulated temperatures during emergence to first flower open (ATEF) and EATEF (effective ATEF) did not respond to the change in temperature. Further experiments illustrated that ATEF was not affected at 22 – 30°C regardless of either same or different day/night temperatures. Finally, ATEF could be used to eliminate the effect of temperature in natural condition, therefore represent photosensitivity exactly better than DEF. Overall results led a conclusion that temperature could affect plant development quantitatively in soybean.

In order to evaluate the photosensitivity comprehensively in *GmWMC* genotypes, we conducted two experiments in the controlled environment. A preliminary experiment was conducted using eight selected genotypes under 8−14 h photoperiod. The lowest ATEF were observed between 8–10 h photoperiod in all genotypes and longest ATEF at 14 h photoperiod. However, we chose 10 h for short photoperiod because it might be better for growth and development, and 13 h for long photoperiod because some genotypes did not open those flowers at 14 h. Under these 13- and 10-h photoperiodic conditions at 28°C, we

tested the ATEF of 82 *GmWMC* genotypes and evaluated their index of photosensitivity of flowering (IPF). Result showed that the ATEF were 560−1372°C under 13 photoperiod and 560− 868°C under 10 h photoperiod. IPF varied from 0.00 to 0.47 in *GmWMC* genotypes. Preliminary experiment also suggested that 10 h photoperiod was suitable condition for relative JGP evaluation. Because 10 h was the photoperiod which induction phase of most genotypes were minimized. Considering the difference in ATEF at 10 h could represent the relative JGP. It varied from 0 to 308°C accumulated temperature (0 – 11 days at 28°C) in *GmWMC* genotypes. Furthermore, there was a positive relationship between IPF and relative JGP, however wide distribution range was also observed, indicating IPF may be moderately independent from relative JGP. Finally, data represented some special traits in some genotypes, namely low IPF with medium relative JGP (Karasumame, Karasumame (Heitou) Black Seed) and high IPF with long relative JGP (Miss 33 Dixi, Karasumame 'Naihou'). These genotypes would be important resources for improving soybean production in tropical areas.

SUMMARY IN JAPANESE

（摘要）

近年熱帯アジア諸国におけるダイズの消費量は著しく増加しているが、域内生産量が少なく自足率は極めて低い。熱帯アジアにおけるダイズ生産は、種々自然環境に制限されるが、中でも短日や高温がダイズの子実収量をもっとも制限する要因になっている。ダイズは短日植物であり短日や高温条件によって早く開花してしまい、その結果栄養成長が不足し、子実収量が制限されてしまう。熱帯の短日条件でも一定の栄養成長を確保するには、日長に強い感受性、あるいは長い基本栄養成長性を持つ品種が有利であるが、それら特性の遺伝的変異についての研究報告は少ない。本研究は、遺伝的背景の広い世界ダイズミニコアコレクション（*GmWMC*）82 系統を用い、ダイズの日長及び温度に対する感受性を解析し、熱帯の短日・高温環境に適するダイズの特性を評価する目的で行った。

まず日長に対する反応性及び生産性を評価するために、ダイズ *GmWMC* 82 系統を佐賀市において長日（春播）及び短日（夏播）条件下で栽培した結果、出芽から開花まで日数（DEF）は長日条件では 23～92 日、短日条件では 19～63 日までに広く変異し、すべての系統において短日条件によって短縮された。同様に開花から結莢始まで日数も短日条件によって短縮されたが、結莢期以降は日長の影響を受けなかった。短日条件下における子実収量も大きく変異したが、子実収量は DEF（r=0.61***）、茎長（r=0.55***）、総節数（r=0.66***）などの栄養成長因子との間には正の相関関係がみられたことから、短日による収量低下の原因は栄養成長不足であることは明らかであった。しかし、圃場では日長や温度の季節変化があり、開花までは長日条件では低温、短日条件では高温であったため、上述の結果は温度による影響もあると考えられた。そこで、日長 12 時間、

昼／夜温それぞれ 25／18℃、28／22℃、33／28℃に制御されたグロスチャンバーで *GmWMC* 系統を栽培した結果、DEF は低温区ほど長かったが、DEF を積算温度、さらには有効積算温度に置換えると、温度による差異はほとんどなくなった。この結果から温度は開花において量的効果しか及ぼさないと推測され、圃場試験においては積算温度を用いれば温度の影響を除去できると考えられた。さらに、熱帯地域を想定した日長10時間（短日）と 13 時間（長日）に設定し、28℃条件下でダイズ *GmWMC* 系統の DEF を調査したところ、長日条件では 20～49 日に変異したが、短日条件では 20～31 日とその変異幅が縮まった。したがって、日長感受性（長日に対する短日での DEF の短縮程度）は 0～0.47 と大きく変異し、同様に圃場における長日条件と短日条件（自然日長はそれぞれ約 15.2 時間と 14.0 時間）の場合（0～0.58）とおおよそ一致したことを明らかにした。ところで、10 時間日長は、ほとんどの系統において最短の DEF を示したため、この条件における DEF の遺伝的変異は、基本栄養成長性に由来するものと考え、ダイズ *GmWMC* 系統の相対的な基本栄養成長性は 28℃では 0～11 日と推定された。日長感受性と基本栄養成長性のいずれも圃場における子実収量とは正の相関関係がみられ、これらの特性は今後の熱帯地域に適するダイズ品種の育成にはもっとも考慮すべき要因であると考えられた。

本研究は、世界各地より集められた異なる遺伝的背景を持つダイズ *GmWMC* 系統の日長や温度に対する多様な変異を明らかにし、特に熱帯地域のダイズ生産の制限要因である日長感受性と基本栄養成長性について詳細に解析したものであり、その成果は今後の熱帯地域のダイズ品種改良に貴重な参考情報となる。

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