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Genotypic differences in soybean yield responses to increasing temperature in a cool climate are related to maturity group

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Abstract

To adapt soybean production to climate change, a thorough understanding of its response to high temperature is required. Modeling studies have predicted that high temperature would shorten the growth period and hence lower seed yield of less day length–sensitive (early-maturing) soybean cultivars, whereas the magnitude of yield reduction by high temperature would be smaller in cultivars with higher day length sensitivity (late-maturing), suggesting that late-maturing cultivars would benefit from a future high-temperature environment. Current mean growing season temperature ranges from 19.4 to 22.6 °C in the northern, cool regions of Japan, which is near or below the reported optimum temperature (22–24 °C) for seed yield. We tested the hypothesis that adaptation by growing late-maturing cultivars will be successful in maintaining seed

yield under a cool climate when temperature is increased during 21st century. We used three Japanese soybean cultivars, early-maturing Yukihomare and late-maturing cultivars Ryuhou and Enrei. Plants were grown over 3 years from June to September (a conventional season) under three temperature regimes, T1 (ambient), T2 (1.8–3.6 °C above ambient), T3 (4.8–5.7 °C above ambient), in a sunlit temperature gradient chamber. The leaf area at the full expansion stage, pod and seed numbers, and seed yield increased at elevated temperature in the late-maturing cultivars but not in the early-maturing one. The photosynthetic rate and effective quantum yield of photosystem II at the flowering stage increased at elevated temperature in all three cultivars. The period from sowing to the beginning of flowering (R1) decreased in all three cultivars at elevated temperature, whereas the period from R1 to the beginning of pod addition and the flowering period were prolonged in the late-maturing cultivars, but not in the early-maturing one. The differential response in post-flowering development in different maturity groups is probably related to the differences in the day length requirements of these cultivars. Our data clearly demonstrate that yield enhancement by increasing temperature in the late-maturing cultivars resulted from both the improvement in sources (leaf area and leaf photosynthesis) and the increase in sink size (number of flowers, pods and seeds) due to the longer flowering period. We conclude that the yield of the late-maturing cultivars sown during the conventional season in the cool regions of Japan will increase during the 21st century.

Keywords

Global warming, High temperature, Maturity group, Soybean, Yield.

Highlights

・Early- and late-maturing soybean cultivars were grown under three temperature regimes.

・High temperature increased seed weight of late- but not early-maturing cultivars.

・The increase in seed weight resulted from an improvement in sources and sinks.

・Larger sinks were attributed to the longer flowering period which occurred under elevated temperature.

Abbreviations

Chl, chlorophyll; CSDL, critical short day length; Φ_{PSII}, effective quantum yield of photosystem II electron transport; F_m, maximum Chl fluorescence of a dark-adapted leaf; F_m', maximum fluorescence in the light-adapted state; F_s, steady-state Chl fluorescence; F_o, initial Chl fluorescence of a dark-adapted leaf; F_v, variable Chl fluorescence of a dark-adapted leaf; LA, leaf area; MG, maturity group; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; PSII, photosystem II; TGC, temperature gradient chamber.

1. Introduction

The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) concluded that (1) by 2012, the globally averaged surface temperature had increased by 0.65–1.06 °C in comparison with 1880; (2) by 2081–2100, it will increase by 0.3–1.7 °C in comparison with 1986–2005 under scenarios of low emission of greenhouse gases and aerosols into the atmosphere and by 2.6–4.8 °C under

high-emission scenarios; (3) daily and seasonal high temperature extremes over most land areas will be more frequent in the future (IPCC, 2013). These changes and variability in climate may have significant impacts on phenology, growth and yield of crops in various regions of the world. Effective strategies to adapt agricultural production to climate change and variability require deep understanding of crop responses to increasing temperature.

Soybean (*Glycine max* [L.] Merr.) is a major source of plant protein and oil, and a major contributor to the world's food supply. In Japan, soybean is an important source of traditional foods and seasonings such as tofu, natto, miso, and soy sauce. To date, the impact of high temperature on soybean production has been assessed by using several approaches. Many experimental studies have shown that temperature is a major determinant of soybean yield. For instance, Sionit et al. (1987) showed that an increase in daytime temperature from 18 °C to 26 °C during the entire growing period increased the seed number and yield. Gibson and Mullen (1996) found that a temperature increase from 30/20 °C (day/night) to 35/30 °C reduces the seed number and size. Recent analysis of long-term data (1976–2006) for the northern Corn Belt of the USA showed that increased temperature during the summer season had a negative impact on soybean yield, causing a 1.6% reduction in yield per 1 °C rise in temperature (Kucharik and Serbin, 2008). Hatfield et al. (2011) also predicted that a 0.8 °C temperature rise would cause a 2.4% decline in soybean yield in the southern USA (current growing season temperature of 26.7 °C), but the same temperature rise would increase soybean yield in the midwestern USA (mean air temperature of 22.5 °C) by approximately 1.7%. On the basis of long-term field experiments (1987–2007) in northeastern China, Zheng et al. (2009) reported that soybean seed yield was increased by 6%–10% per 1 °C rise in

mean daily maximum temperature during seed filling. Thus, the impact of temperature increase on soybean yield is highly dependent on the baseline temperature.

Process-based crop growth models are effective tools to assess the impact of climate changes on development, growth and yield of crop plants. Several crop models have been developed for soybean (e.g., Sinclair-Soybean, Sinclair, 1986; CROPGRO-Soybean, Boote et al., 1998; SoySim, Setiyono et al., 2010). Most crop models parameterize phenology by defining the main developmental stages (basic vegetative phase, photoperiod-sensitive phase, post-inductive phase) sensitive to temperature, photoperiod, or both and phenology contributes to dry matter accumulation and partitioning and to seed yield (Craufurd et al, 2013). Soybean is a short-day plant, and longer day length slows its development. The development rate is largely determined by cultivar-specific day length requirements. Unlike soybean late-maturing cultivars, early-maturing cultivars are nearly insensitive to day length. The CROPGRO-Soybean model predicts that soybean seed yield is highest at 22–24 °C mean temperature. By using this model, Boote (2011) predicted that a projected 2 °C temperature rise would cause earlier flowering and pod set, resulting in shorter vegetative growth, smaller leaf area (LA) and lower seed yields in less day length–sensitive cultivars (early-maturing; lower ratings according to the maturity group [MG] classification in the USA) in the midwestern USA at the current growing season temperature of 22.5 °C. Furthermore, he predicted that the magnitude of yield reduction by rising temperature would be smaller in cultivars with higher day length sensitivity (late-maturing; higher MG ratings), suggesting that late-maturing cultivars would benefit from a future high-temperature environment. Similarly, some studies suggested

that longer vegetative growth is an effective strategy for adaptation to high temperature, which shortens the growth period and hence lowers crop yields (Challinor and Wheeler, 2008; Tao and Zhang, 2010).

A temperature gradient chamber (TGC) is a valuable experimental tool to assess the impact of projected global warming (Horie et al., 1995). A TGC can be used to evaluate crop responses to season-long high temperatures under field-like conditions. Some experiments on soybean conducted in the southwestern warm regions of Japan used TGC. Oh-e et al. (2007) reported that soybean yield was increased by high mean daily temperatures (27–31 °C) during seed filling, but reduced by temperatures above 31 °C. Tacarindua et al. (2012, 2013) reported that an increase in temperature during the growing season from 26 to 30 °C reduced leaf photosynthesis, seed yield, seed number, pod number, and single seed weight.

Current mean temperature during the growing season (June to September) between 1981 and 2010 ranges from 19.4 to 22.6 °C at meteorological observatories in the northern regions of Japan (Table A.1), which is near or below the optimum temperature for soybean yield. Based on these facts, we hypothesized that the anticipated increase in temperature during this century may have a positive effect on soybean production in cool climates, and that the late-maturing soybean cultivars would be superior to the early-maturing ones under future warming. We investigated the effects of season-long high temperature on seed yield of three Japanese soybean cultivars of different MGs for three consecutive years (2011, 2012 and 2013) by using a TGC. To understand the reason for differential seed yield responses of soybean cultivars, we focused on responses of the yield components, phenology, flowering period, flower number, leaf

photosynthesis, and chlorophyll (Chl) fluorescence. Our data clearly demonstrate that the genotypic differences in soybean yield responses to increasing temperature are related to MG in a cool region of northern Japan. In addition, yield enhancement by elevated temperature in the late-maturing soybean cultivars was attributed to the larger sources (LA and leaf photosynthesis) and sinks (numbers of flowers, pods and seeds).

2. Materials and methods

2.1 Plant materials and growth conditions

The experiments were conducted at the NARO Tohoku Agricultural Research Center in Morioka, Japan (39°44'N, 141°7'E) from June to October for three consecutive years (2011, 2012, and 2013). Soybean cultivars used were Yukihomare (early-maturing; Japanese ecological type Ib [see below]; equivalent to MG II or earlier because its period from emergence to the beginning of maturity [R7] is 3 days longer than in cv. Mandarin of MG I), Ryuhou and Enrei (late-maturing; Japanese ecological type IIIb, equivalent to MG IV). Japanese soybean cultivars are classified into nine ecological types (Ia, Ib, IIa, IIb, IIc, IIIb, IIIc, Ivc, and Vc) according to a combination of the days from germination to flowering (I–V) and the days from flowering to maturity (a–c), in ascending order (Fukui and Arai, 1951). Yukihomare, Ryuhou, and Enrei are widely cultivated in the Hokkaido region (northern Japan), the Tohoku region (northern Japan), and the Kantou and Hokuriku regions (central Japan), respectively. All cultivars were of the determinate type. Prior to sowing, each pot was supplied with 10 g of compound fertilizer, which included 0.3, 1.0 and 1.0 g of N, P_2O_5 and K_2O, respectively, 5 g of fused magnesium phosphate and 5 g of dolomitic lime, according to standard regional

agronomic practices. The soil was a low humic Andisol containing 6.57% of C and 0.62% of N. Pot size was 5 L in 2011 and 2012 (4 kg of soil), and 10 L in 2013 (8 kg of soil). Seeds were inoculated with *Bradyrhizobium japonicum* (Mamezou, Tokachi-Nokyouren, Japan) and sown (three to five seeds per pot) on 7 June 2011, 2012, and 2013. After seedling emergence, plants were thinned to one plant per pot and grown until harvest in a TGC, i.e., a naturally sunlit greenhouse (6 m wide, 30 m long and 3 m high) with an air inlet at one end and exhaust fans at the other end (see Okada et al. [2000] for a detailed description). The air in the TGC flowed continuously from the inlet to the exhaust fans. A temperature gradient inside the TGC were continuously maintained along the longitudinal axis by cooling the air with an air conditioner at the inlet end, warming the air by solar radiation or a supplemental heat input (a heater and air ducts) at the outlet end, or both. To create a point where air temperature was equivalent to that outside, the air temperature at the inlet end was maintained 2–3 °C below the outside temperature by cooling. Three regimes were set along the temperature gradient: low (T1), middle (T2), and high temperature (T3). Air temperature in T1 was equivalent to that outside. The temperature difference between T1 and T3 was targeted to approximately 5~6 °C by regulating the fans and heat sources. Eight to ten pots per cultivar were arranged without mutual shading in each regime. Pots were rotated at 7-day intervals to minimize the effects of environmental differences. Pt-100 resistance thermometers with an aspirated double-tube radiation shield were installed to monitor air temperature in each regime and periodically moved upward to match plant height. Air temperature was measured every 5 s, and the means were recorded every 30 min and 24 h by a datalogger (CR1000; Campbell Sci. Inc., Logan, USA). Daily mean solar radiation during the experiments was recorded at a weather station of the research center

located 1 km NNE from the TGC. Plants were irrigated with tap water once or twice a day to maintain soil water near field capacity level. Pesticides were applied when necessary. Weeding was carried out by hand weekly from sowing to harvesting.

2.2 Measurements of phenology, growth, and yield

The phenology of four to five plants of each cultivar in each regime was surveyed once a day, and the date of the beginning of flowering (R1; defined as the date when 50% of the plants had at least one flower), the date of the beginning of pod setting (R3; defined as the date when 50% of the plants had at least one 5-mm-long pod at one of the four uppermost nodes), and the date of the beginning of maturity (R7, defined as the date when 50% of the plants had one mature pod) were recorded according to the definition of growth stages proposed by Fehr and Caviness (1971). When the uppermost leaf on the main stem was fully expanded, the total green leaf area of four or five plants of each cultivar in each regime was measured with an automatic area meter (AAM-9; Hayashi Denko Co. Ltd., Tokyo, Japan). During the flowering period in 2012 and 2013, the number of newly opened flowers of each cultivar in each regime was counted at 1- or 2-day intervals. The number of days from R1 to the termination of flowering (i.e., after the last flower had opened) was defined as the flowering period. At harvest maturity, the above-ground parts of four or five plants per regime were harvested, and the components (leaves, stems, pod shells, and seeds) were separated. Simultaneously, the numbers of nodes on the main stem, branches, fertile pods, and seeds per plant were recorded. The dry weight of seeds was measured after oven-drying at 80°C for 5 days. Seed yield per plant was adjusted to 15% moisture content, and the mean single-seed weight was determined by dividing the seed yield per plant by the number of seeds. Pod

setting ratio was determined by dividing the number of fertile pods by the total number of opened flowers.

2.3 Measurements of photosynthesis and Chl fluorescence

Photosynthetic gas exchange and Chl fluorescence of the most recently expanded terminal leaflet of four different plants from each T1 and T3 was measured at 2 weeks after R1. Photosynthetic gas exchange was measured in all 3 years, whereas Chl fluorescence was measured in 2012 and 2013. Light-saturated photosynthetic rate and stomatal conductance were measured between 09:00 and 12:00 h using a portable photosynthesis measurement system (LI-6400; Li-Cor Inc., Lincoln, NE, USA). The CO_2 concentration ($[CO_2]$) in the air entering the leaf chamber was adjusted to 390–400 μmol mol^{-1}. Air temperature in the chamber matched that of the respective regime. The relative humidity of air entering the chamber was adjusted to approximately 60%. The photosynthetic photon flux density (PPFD) inside the chamber was set at 1500 μmol m^{-2} s^{-1} by means of an internal light source. At the same time, Chl fluorescence of photosystem II (PSII) was measured using the LI-6400 system with an integrated fluorescence chamber head (LI6400-40; Li-Cor Inc.). The conditions in the leaf chamber were as above. Using a leaf that had been dark-adapted for 20 min, the initial fluorescence (F_0) under non-photosynthetic conditions was determined with a measuring beam of low intensity. The maximal fluorescence (F_m) was subsequently measured by applying a saturating pulse of ~8000 μmol m^{-2} s^{-1} to reduce all PSII centers. The leaf was then continuously irradiated by actinic light (PPFD of 1500 μmol m^{-2} s^{-1}). After the steady-state fluorescence (F_s) was recorded, a saturation pulse was applied to determine the maximum fluorescence in the light-adapted state (F_m'). The

following parameters were calculated: maximum quantum yield of PSII, $F_v/F_m = (F_m - F_0)/F_m$ (van Kooten and Snel, 1990); quantum yield of PSII electron transport, $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty et al., 1989); and non-photochemical quenching, $NPQ = (F_m - F_m')/ F_m'$ (Bilger and Björkman, 1990). After the measurements, the mean of three readings from the SPAD meter (SPAD-502; Konica Minolta Sensing Co., Osaka, Japan) was obtained from the middle portions of the leaves.

2.4 Statistical analysis

To test the significance of differences for temperature regimes and cultivars and their interaction, we used two-way analysis of variance (ANOVA) on the data for phenology, yield, and leaf photosynthesis from three replications (years) and Chl fluorescence, flowering period, the number of opened flowers, and the pod setting ratio from two replications (years). Analysis was conducted for mean values of four or five plants in each year. When the ANOVA produced a significant result, Fisher's LSD test for significant differences between means was performed. All statistical analyses were performed with SPSS statistics software (SPSS ver. 22.0; IBM Co., New York, NY, USA).

3. Results

3.1 Meteorological conditions during the growing season

In T1, the 4-month mean temperature in 2011 and 2013 was similar to the 30-year mean outside temperature, but was approximately 1 °C higher in 2012 (Table 1), with the largest difference in September (3.4 °C above the 30-year mean). The 4-month mean air temperature in T1 ranged between 20.1 and 21.5 °C, 4.8–5.7 °C lower than in T3;

the difference between T1 and T2 was 1.8–3.6°C. The 4-month mean solar radiation in 2011 and 2013 was close to the 30-year mean values, but was higher than average in 2012 (Table 1).

3.2 Yield, yield components and vegetative growth

The early-maturing cultivar Yukihomare had significantly lower values of all parameters related to yield and vegetative growth than the late-maturing cultivars Ryuhou and Enrei (Table 2). A significant ($p < 0.05$) positive effect of temperature was found for all parameters except the branch number. Moreover, there were significant temperature × cultivar interactions ($p < 0.05$) for seed yield, seed number, and pod number. Increasing temperature significantly increased seed yield in Ryuhou (by 10% in T2 and 18% in T3 as compared with T1) and Enrei (by 17% in T2 and 18% in T3), but not in Yukihomare. Both seed number and pod number increased with temperature rise in Ryuhou (by 14% and 10%, respectively, for T2, and by 30% and 35% for T3 as compared with T1) and Enrei (by 18% and 21%, respectively, for T2, and by 30% and 35% for T3), but not in Yukihomare. A similar trend was observed for LA of all cultivars. Temperature rise from T1 to T3 significantly reduced the single seed weight ($p < 0.05$) and the node numbers on the main stem ($p < 0.05$) in all cultivars.

3.3 Phenology

The number of days between the main developmental stages was significantly smaller in Yukihomare than in Ryuhou and Enrei (Table 3), which corresponded to their MGs. The periods from sowing to R1 and from R3 to R7 decreased significantly with increasing temperature ($p < 0.001$ and $p < 0.01$, respectively) without a significant

temperature × cultivar interaction (Table 3). The R3–R7 period was less affected by temperature than the period from sowing to R1: the latter was 7–11 days shorter in T3 than in T1, whereas the former was 2–5 days shorter. In contrast, the R1–R3 period was significantly longer ($p < 0.001$) in T3 than in T1, and there was a significant temperature × cultivar interaction ($p < 0.001$); the difference between T1 and T3 was grater in Enrei (10 days) and Ryuhou (7 days) than in Yukihomare (1 day). A significant temperature × cultivar interaction ($p < 0.01$) was found for the R1–R7 period. In Enrei and Ryuhou, it was 7 and 3 days longer in T3 than in T1, respectively, whereas in Yukihomare it was 3 days shorter in T3 than in T1. As a consequence, higher temperature significantly shortened the period from sowing to R7 ($p < 0.001$), with a significant interaction with the cultivar ($p < 0.05$). The magnitude of this reduction differed among the cultivars, and was largest for Yukihomare (7 days in T2 and 11 days in T3 as compared with T1), followed by Ryuhou (6 and 7 days) and Enrei (5 and 4 days).

3.4 Flowering period, flower number and pod setting

The length of the flowering period and the total number of opened flowers in Yukihomare were significantly ($p < 0.001$) lower than those in Ryuhou and Enrei (Fig. 1A and B). The ANOVA results showed that rising temperature significantly prolonged the flowering period ($p < 0.001$) and significantly increased the number of opened flowers ($p < 0.001$), with significant temperature × cultivar interactions ($p < 0.05$). In Ryuhou and Enrei, these parameters were higher in T3 than in T2 and T1, whereas in Yukihomare they were similar in all three regimes. The effect of temperature and the interaction between temperature and cultivar were not significant for the pod setting

ratio (Fig. 1C).

3.5 Photosynthesis and Chl fluorescence

Photosynthetic rate, Φ_{PSII}, and NPQ were significantly affected by both temperature ($p < 0.01$) and cultivar ($p < 0.01$) (Fig. 2A, E, and F). Higher growth temperature increased the photosynthetic rate and Φ_{PSII} but decreased NPQ in all three cultivars, without significant temperature × cultivar interactions. However, there were no individual effects of temperature and cultivar, or temperature × cultivar interactions, for stomatal conductance and F_v/F_m (Fig. 2B and D). The SPAD readings were significantly affected by cultivar ($p < 0.001$) but not by temperature (Fig. 2C), and there was no temperature × cultivar interaction for SPAD values.

4. Discussion

The objective of this study was to test the hypothesis that adaptation by growing late-maturing soybean cultivars will be successful in maintaining seed yields under a cool climate when temperature is increased during 21st century. Our hypothesis was partially validated, as discussed below.

4.1 Differences in the temperature responses of seed yield

It is widely accepted that there are inter- and intra-specific differences in the temperature responses for both vegetative biomass and grain yield in some crops. For instance, two ecotypes of rice, *indica* and *japonica*, have warmer and cooler optimum temperatures, respectively (Craufurd et al., 2013). In soybean, the responses of early

reproductive growth to low night temperature (<16 °C) clearly differed between cultivars adapted to tropical and temperate climates (Lawn and Hume, 1985). Genetic variation in the responses of traits contributing to pod setting, namely pollen germination and pollen tube growth, to extremely high temperature (38/30 °C) has been documented in soybean (Salem et al., 2007). However, information about the differences among soybean cultivars in the response to moderate temperature for seed yield is limited. Although the CROPGRO-Soybean suggests that the optimum temperature for maximizing seed yield is between 22 and 24 °C (Boote et al., 1998, Boote, 2011), our study revealed that an increase in growth temperature to a value in the range of 20–27 °C increases yield in the late-maturing cultivars, but not in the early-maturing one. These increases in seed yield in T2 and T3 regimes compared with T1 in the late-maturing cultivars were attributed to the improvement in sources (LA and leaf photosynthesis) and large sinks (number of flowers, pods and seeds) due to the extension of the flowering period. Baker et al. (1989) also reported that increase in seed number and reduction in seed size for soybean with rising temperature from 26/19 °C to 31/24 °C, which is agreement with our results.

4.2 Differences in the temperature responses of LA and leaf photosynthesis

The importance of LA for yield has been recognized in crop plants. Temperature directly affects LA expansion. Significant increases in LA with increasing temperature were found in the two late-maturing cultivars but not in the early-maturing one (Table 2). Maximum LA is a product of the number of days from emergence to full expansion and average daily LA expansion rate during the expansion. We observed that the number of days to full expansion was smaller (data not shown) and the LA expansion

rate was higher (Fig. A. 1) in T2 and T3 than in T1 in all cultivars. However, the increase in the LA expansion rate was somewhat smaller in the early-maturing cultivar than in the late-maturing ones. Temperature did not affect the branch number (Table 2). Our data indicate that a higher LA expansion rate at higher temperature may lead to an increase in total LA in the late-maturing cultivars.

In addition to LA, leaf photosynthesis is fundamental for dry matter production and yield. In soybean, photosynthesis at the canopy level is weakly affected by daytime temperature over a broad range (26–36 °C; Campbell et al., 1990). In our study, the average temperature during the measurement time (09:00 – 12:00 h) was 25–27 °C in T1 and 30–32 °C in T3. T3 increased the photosynthetic rate of all cultivars (Fig. 3A), which could not be attributed to the response of stomatal conductance and Chl content (SPAD readings) (Fig. 3B and C). Although PSII function was reported to be temperature-sensitive and photosynthesis in soybean to be down-regulated at excessively high temperature (38/28 °C) (Djanaguiraman et al., 2011), moderately high temperature had no effect on F_v/F_m in this study (Fig. 3D). F_v/F_m is widely used as an indicator of PSII function (van Kooten and Snel, 1990). Φ_{PSII} represents the fraction of energy used for photosynthetic electron transport in the chloroplast thylakoid membrane, and the most of Φ_{PSII} could be the electron flux to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)–mediated carboxylation (Genty et al., 1989). It is suggested that the higher photosynthetic rate at higher temperature resulted from the increased activity of Rubisco–mediated carboxylation rather than from the enhanced PSII function. Canopy photosynthesis during the early reproductive stage is essential for determining the seed number in soybean (Kokubun and Watanabe, 1981; Egli, 2010). In our study, the greater seed number at higher temperature in the late-maturing cultivars

was most likely due to both large LA and high leaf photosynthetic rate.

4.3 Differences in temperature responses of phenology, flowering period, and the number of flowers, pods, and seeds

Floral growth is regulated mainly by temperature, but floral initiation is primarily a photoperiodic response (Hesketh et al., 1973; Setiyono et al., 2010). In soybean, the period from emergence to R1 decreased with increasing temperatures up to 36/26 °C, but increased at higher temperatures (Thomas et al., 2010). Although the effect of temperature on post-flowering development was not significant, the period from R5 (beginning of seed filling) to R7 and the effective seed-filling period were shortest at 28/18 °C and 32/22 °C, respectively (Thomas et al., 2010). Seddigh et al. (1989) reported that the R1–R7 and R3–R7 periods were unaffected by an increase of night temperature from 10 to 24 °C. Likewise, Hesketh et al. (1973) found that seed-filling period was almost unaffected by temperature in the range from 21 to 30 °C. Our results show that the periods from sowing to R1 were significantly shortened at high temperature in all three cultivars (Table 3). The R1–R3 and R1–R7 periods responded differentially to increasing temperature: the R1–R3 and the R1–R7 periods were lengthened in the late-maturing cultivars but not in the early-maturing one. One possible explanation for these differential responses of post-flowering development to increasing temperature is the cultivar-specific day length requirement as discussed in a next section.

Temperature above 32 °C reportedly reduces flower initiation and growth in soybean (Borthwick and Parker, 1940). The temperature range set in our study was not high enough to inhibit flower growth, but a longer flowering period was observed in the

late-maturing cultivars under high temperature (Fig. 2A). In both late-maturing cultivars, the number of opened flowers increased rapidly during 10 days after R1 in all regimes, but then continued to increase in T3, whereas this increase was weaker in T1 and T2 (Fig. A. 2). On the other hand, temperature did not affect the flower number in the early-maturing cultivar. Interestingly, changes in the flowering period were consistent with those in the R1–R3 period. As a consequence, high temperature increased the total number of opened flowers in late-maturing cultivars. Some reports showed that the pod and seed numbers in soybean increase when the post-flowering phase is longer (Summerfield et al., 1998; Kantolic and Slafer, 2007). Therefore, the increased number of opened flowers at higher temperatures could result in an increase in the pod and seed numbers in late-maturing cultivars. Here, we have confirmed that the increase in seed yield at high temperature in the late-maturing cultivars involved the increase in the seed, pod and opened flower numbers, which resulted from the extension of the flowering period.

4.3 Differential responses of post-flowering development may be related to the day length requirements

There is ample evidence that longer photoperiod slows down the post-flowering development of a wide range of soybean cultivars. Summerfield et al. (1998) reported that long photoperiod increased the periods from R1 to the end of flowering and from R1 to R7 in indeterminate cultivars of various MGs. Field experiments with indeterminate soybean demonstrated that extension of the photoperiod after flowering increases the duration of the post-flowering phase (Kantolic and Slafer, 2007), and that

this effect was more evident in the late-maturing lines (Kumudini et al. 2007). In the crop growth models, the reciprocal of the R1–R7 period (divided into R1–R3, R1–R5, and R5–R7) was a linear-plateau function of photoperiod under optimum temperatures (Boote et al., 1998, Soltani and Sinclair, 2012). According to these models, the developmental rate of short-day plants is maximal under shorter photoperiods. The threshold photoperiod (below which the development rate is maximal) and the decline in the developmental rate with increasing photoperiod are defined as the critical short-day length (CSDL) and photoperiod sensitivity, respectively. Lower MG rating corresponds to greater CSDL and lower photoperiod sensitivity (Boote et al., 1998, Soltani and Sinclair, 2012). Based on this assumption, under a given day length the early-maturing cultivar (Yukihomare) with a greater CSDL can achieve R3 and complete flowering faster than the late-maturing cultivars with lower CSDL. According to the logistic model (Horie and Nakagawa, 1990), the CSDL for the R1–R7 period was greater in the early-maturing cultivar than in the late-maturing ones (our unpublished results). Therefore, the differential response of post-flowering development is probably related to the differences in the day length requirements of these cultivars. No differences in the temperature response of seed yield between different MGs are expected under short-day conditions (for example, in the case of late sowing).

Some crop growth models considered the elevated-temperature effect on the delay of post-flowering phase of soybean (Boote, 1997; Setiyono et al., 2007). Indeed, Thomas et al. (2010) reported that the rate of post-flowering phase from R5 to R7 of Bragg (MG VII) was fastest at 28/18 (mean 23 °C) under both spring (shorter day length) and fall (longer ones), suggesting that elevated temperature by itself can cause prolonged the post-flowering phase of late-maturing soybean cultivars under short-day condition.

Further investigation using cultivars with various MGs under the various sowing dates is needed to elucidate the interactions between temperature, cultivar and day length in regulation of post-flowering development.

In soybean, nine major loci, E1–E8 and J, control time to flowering and maturity (reviewed by Watanabe et al., 2012). Except for E6, their dominant alleles increase time to flowering and maturity in response to long photoperiod. Recently, Xu et al. (2013) suggested that *Phytochrome A* genes (E3 and E4) regulate the response of pod addition to photoperiod. Using DNA markers and sequencing, genotypes at the E1–E4 loci have been determined for many accessions covering several Japanese ecological types (Tsubokura et al., 2014). Near-isogenic lines of Enrei for the dominant alleles E2 and E3 have been developed by backcrossing followed by marker-assisted selection (Yamada et al., 2012). Further experiments taking into account this genetic information are needed to test whether late-maturing cultivars benefit from elevated temperature and to clarify the “optimum genotypes” when increasing temperature under cool climates.

Conclusions

Our 3-year TGC experiments clearly demonstrate that an increase in growth temperature to a value in the range of 20–27 °C increases yield in the late-maturing soybean cultivars sown at a normal time (early June). Yield enhancement resulted from the improvement in sources (LA and leaf photosynthesis) and large sinks (number of flowers, pods and seeds). The large sinks could be involved in the extension of the R1–R3 period and flowering period at higher temperatures. The 4-month (June to September) mean temperatures in the northern cool areas of Japan (anticipated by MIROC-H under the A1B scenario; Iizumi et al., 2012) are predicted to range from 23.7

to 26.8 °C in the far future (2062–2091). To accurately predict future crop production, it is necessary to assess the impacts not only of future warming, but also of elevated [CO_2]. Our previous study showed that elevated [CO_2] (current +200 μmol mol^{-1}) increases the seed yield of Enrei in Morioka (22–25 °C) (Kumagai et al., 2012). Therefore, we predict that seed yield of late-maturing cultivars (MG IV) sown at normal season in the northern part of Japan will increase during the 21st century.

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Appendix A. Supplementary data

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Figure captions

Fig. 1. Flowering period (A), the number of opened flowers per plant (B) and the pod setting ratio (C) of three soybean cultivars grown under three temperature regimes (T1, T2 and T3). Data are mean ± SE (n = 2 years). The same letters within each cultivar indicate no significant difference as determined by Fisher's LSD test at the 5% level. ANOVA results: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

Fig. 2. Photosynthetic rate (A), stomatal conductance (B), SPAD readings (C), maximum quantum efficiency of PSII (F_v/F_m; D), effective quantum yield of PSII (Φ_{PSII}; E) and non-photochemical quenching (NPQ; F) of three soybean cultivars grown under two temperature regimes (T1 and T3). Measurements were made 2 weeks after R1. Data are mean ± SE (n = 2 or 3 years). The same letters within each cultivar indicate no significant difference as determined by Fisher's LSD test at the 5% level. ANOVA results: ***, $P < 0.001$; **, $P < 0.01$; ns, not significant.

Tables

Table 1. Mean monthly air temperature and solar radiation during growth under three different temperature regimes (T1, T2 and T3) in 2011, 2012, and 2013.

Parameter and year	Temperature regime	June	July	August	September	4-month mean
Air temperature (°C)						
30-year mean	Outside	18.3	21.8	23.4	18.7	20.6
2011	T1	18.4	21.7	21.6	18.6	20.1
	T2	22.2	25.5	25.1	21.9	23.7
	T3	24.5	27.6	27.2	24.1	25.8
2012	T1	18.1	21.8	24.2	22.1	21.5
	T2	20.0	23.6	26.1	23.9	23.4
	T3	23.1	26.6	29.0	26.7	26.3
2013	T1	19.4	20.5	22.3	19.2	20.4
	T2	21.4	22.4	24.2	20.9	22.2
	T3	24.6	25.4	27.1	23.8	25.2
Solar radiation (MJ m^{-2} d^{-1})						

30-year mean	15.7	14.5	14.9	11.3	14.1
2011	17.9	18.6	14.0	10.8	14.6
2012	18.0	16.9	18.7	14.1	15.8
2013	19.1	12.0	16.6	12.2	13.8

Table 2. Yield and yield components of three soybean cultivars grown under three temperature regimes (T1, T2 and T3).

Cultivar	Temperature regime	Seed yield (g plant^{-1})	Seed number (plant^{-1})	Pod number (plant^{-1})	Single seed weight (mg)	Branch number (plant^{-1})	Node number on the main stem	Leaf area (m^2 plant^{-1})
Enrei	T1	63.9a	187.8a	94.0a	340.9a	8.4	15.9a	1.23a
	T2	75.1b	221.5b	114.1b	345.6a	8.8	15.3ab	1.48b
	T3	74.9b	233.2b	133.1c	325.3b	8.8	14.9b	1.50b
Ryuhou	T1	63.4a	170.4a	92.0a	374.4a	7.7	15.2a	0.77a
	T2	69.6ab	194.8ab	100.9a	363.1a	7.9	14.7a	0.92ab
	T3	74.8b	222.0b	123.8b	341.1b	7.6	13.6b	1.03b
Yukihomare	T1	38.3a	113.9a	60.4a	335.7a	4.6	9.7a	0.31a
	T2	39.7a	131.1a	68.9a	302.8b	4.6	9.4ab	0.39a
	T3	31.5a	114.9a	60.7a	275.6b	5.1	9.0b	0.30a
ANOVA results	Temperature (T)	*	**	***	*	ns	*	*
	Cultivar (C)	***	***	***	***	***	***	***

T×C	*	*	*	ns	ns	ns	*

Values are means (n = 3 years). Values followed by the same letters in each cultivar were not significantly different as determined by Fisher's LSD test at the 5% level. ANOVA results: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

Table 3. Phenology of three soybean cultivars grown under three temperature regimes (T1, T2 and T3).

		Days				
Cultivar	Temperature regime	Sowing–R1	R1–R3	R3–R7	R1–R7	Sowing–R7
Enrei	T1	55a	11a	50a	61a	116a
	T2	50b	15b	47a	61a	111b
	T3	44c	21c	47a	68b	112ab
Ryuhou	T1	53a	8a	50a	58a	111a
	T2	46b	10a	50a	59ab	105b
	T3	42c	13b	48a	61b	103b
Yukihomare	T1	38a	5a	47a	51a	89a
	T2	34b	5a	44ab	49a	82b
	T3	31c	6a	42b	48b	78c
ANOVA results	Temperature (T)	***	***	**	ns	***
	Cultivar (C)	***	***	***	**	***
	T×C	ns	***	ns	**	*

Values are means (n = 3 years). Values followed by the same letters in each cultivar were not significantly different as determined by Fisher's LSD test at the 5% level. ANOVA results: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

Table A.1. Recent and future 4-month (June−September) mean temperature at meteorological observatories in the northern regions of Japan.

City	1981−2010	2062−2091
Sapporo	19.4	23.7
Aomori	20.2	24.8
Morioka	20.6	24.6
Akita	21.9	25.8
Sendai	21.4	25.2
Yamagata	22.0	25.7
Fukushima	22.6	26.8
Mean	21.1	25.2

Future temperatures are indicated according to a global climate model (MIROC-H) under the A1B scenarios in the ELPIS-JP data set (Iizumi et al., 2012).

Fig. A.1. Total number of opened flowers during the flowering periods in three soybean cultivars grown under three temperature regimes (T1, T2 and T3) in 2012 and 2013. Data are means of 4 or 5 plants.

Fig. A.2. Leaf expansion rate of three soybean cultivars grown under three temperature regimes (T1, T2 and T3) in 2012. Data are mean ± SE (n = 4 plants). Leaf expansion rate was calculated as the linear coefficient (slope) of the leaf area as a function of days after sowing (DAS).

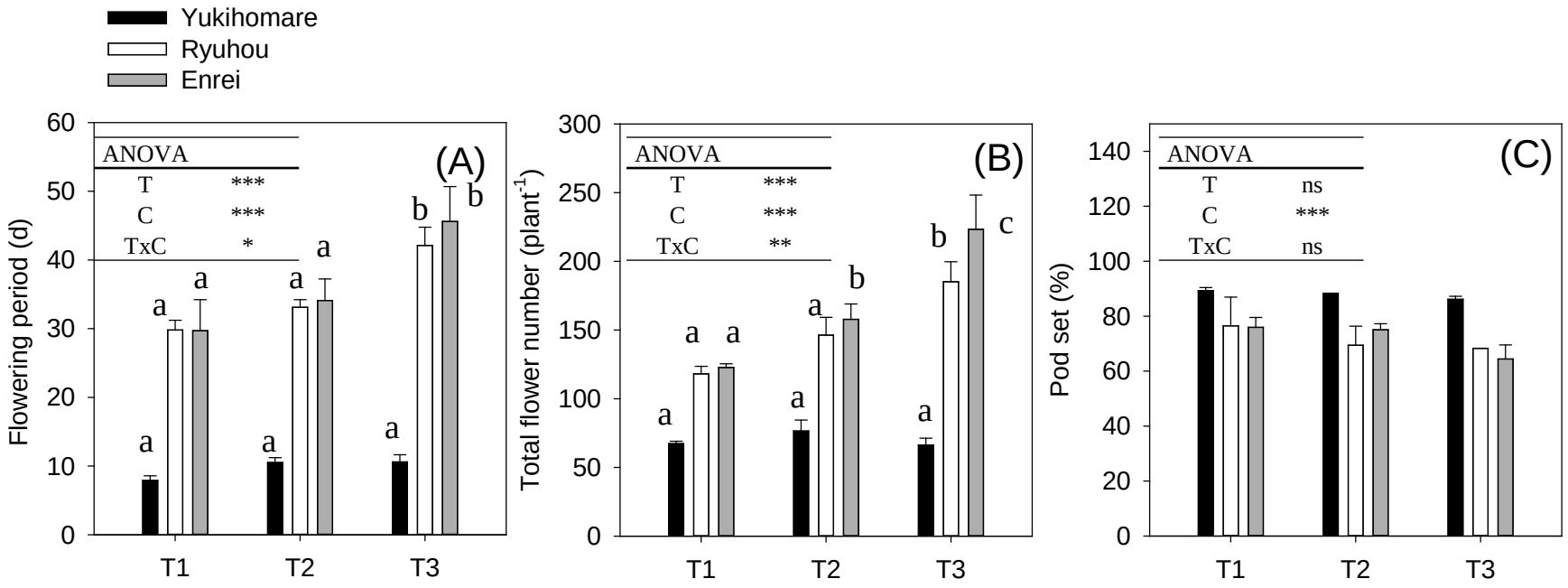

Yukihomare
Ryuhou
Enrei
Flowering period (d)
ANOVA
T ***
C ***
TxC *
(A)
a a a
a a a
b b
T1 T2 T3
Total flower number (plant-1)
ANOVA
T ***
C ***
TxC **
(B)
a a a
a a b
a b c
T1 T2 T3
Pod set (%)
ANOVA
T ns
C ***
TxC ns
(C)
T1 T2 T3

Fig 1.

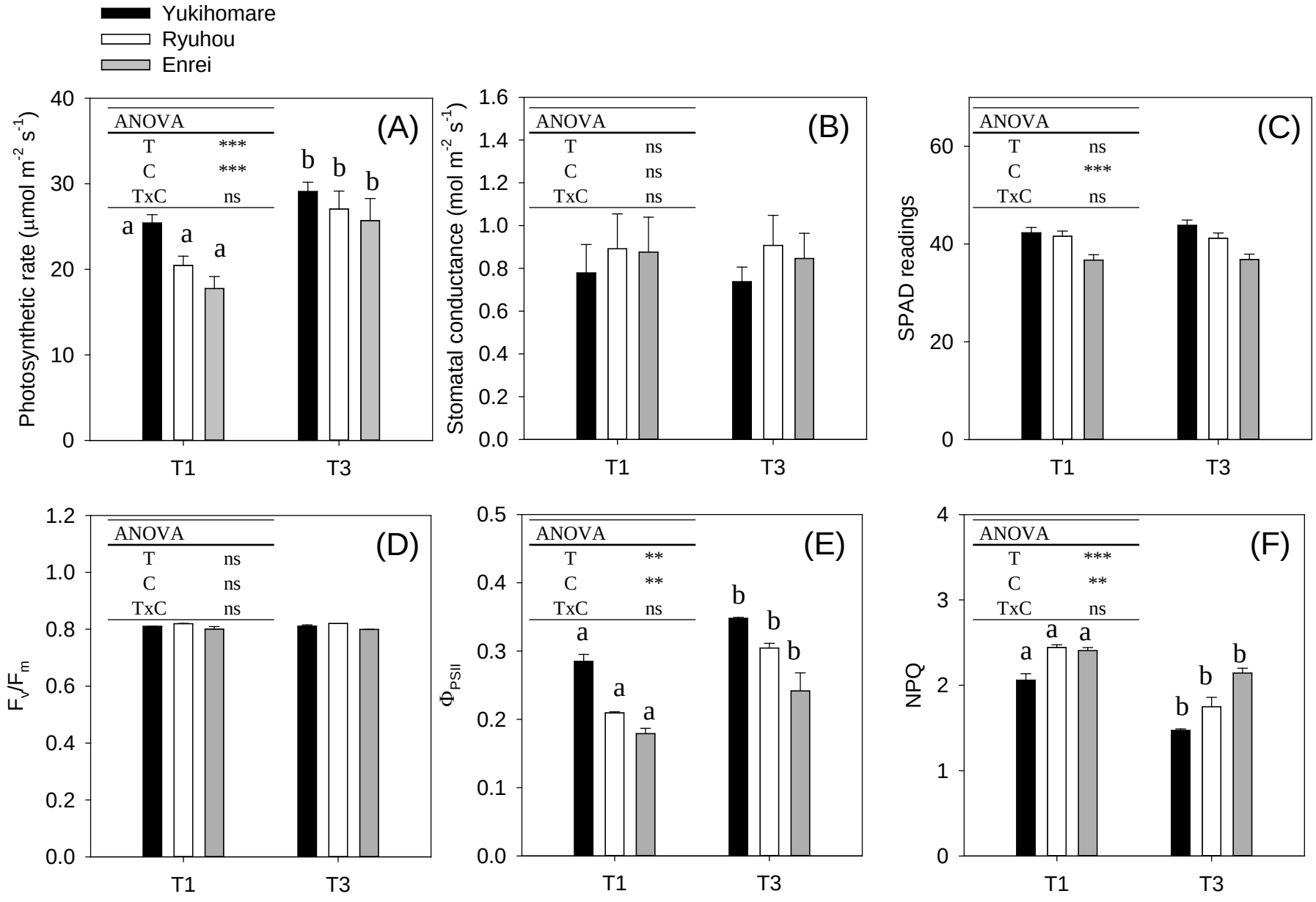

Yukihomare
Ryuhou
Enrei
(A)
Photosynthetic rate (μmol m-2 s-1)
ANOVA
T ***
C ***
TxC ns
a a a b b b
T1 T3
(B)
Stomatal conductance (mol m-2 s-1)
ANOVA
T ns
C ns
TxC ns
T1 T3
(C)
SPAD readings
ANOVA
T ns
C ***
TxC ns
T1 T3
(D)
FV/Fm
ANOVA
T ns
C ns
TxC ns
T1 T3
(E)
ΦPSII
ANOVA
T **
C **
TxC ns
a a a b b b
T1 T3
(F)
NPQ
ANOVA
T ***
C **
TxC ns
a a a b b b
T1 T3

Fig 2.