Plant Biotechnology 33, 323–331 (2016)
DOI: 10.5511/plantbiotechnology.16.0622a

Note

Density effects on late flowering mutants of *Arabidopsis thaliana* under continuous light

Kiwako Yamamoto[1,†], Kei Takahashi[1,†], Miyuki Hara[1,2], Kana Miyata[1], Ryosuke Hayama[1], Tsuyoshi Mizoguchi[1,*]

[1]Department of Natural Sciences, International Christian University (ICU), Osawa 3-10-2, Mitaka, Tokyo 181-8585, Japan;
[2]Gene Research Center, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan
*E-mail: mtsuyoshi@icu.ac.jp Tel: +81-422-33-3247

Received February 18, 2016; accepted June 22, 2016 (Edited by T. Demura)

Abstract In general, plant growth is inhibited under high-density conditions, while it is promoted under low-density conditions. This is known as the "density effect". Growing plants at high densities is often associated with an accelerated flowering time. Three major pathways [the long day (LD), gibberellic acid (GA), and autonomous/vernalization pathways] are known to play important roles in the control of flowering time. Circadian clock genes, namely, *LHY*, *CCA1*, *GI*, and *ELF3*, regulate the LD pathway. GAI and FCA control flowering via GA and autonomous pathways, respectively. The density effect on plant size is caused by specific factors such as the amount of nutrition obtained from the soil and touch frequency among plants. However, the molecular mechanism underlying the acceleration of flowering time due to density effects remains unclear. Here, we show the density effects on three Brassicaceae plants, namely, *Brassica rapa* var. *nipposinica*, *Brassica napus*, and *Brassica chinensis* f. *honsaitai*. They showed shorter stems and leaves when grown at high densities on soil under continuous light (LL). Shorter stems and leaves, as well as accelerated flowering times, were observed when a model plant, *Arabidopsis thaliana*, was grown under the same conditions. Unexpectedly, *ethylene insensitive 2* (*ein2*) showed no differences in density effects in our experiments. The acceleration of flowering at higher densities was largely suppressed by *gai*, but not by *gi*, *lhy;cca1*, or *fca*. These results suggest that the promotion of flowering (as a density effect) is likely dependent on the GA pathway, but not the LD or autonomous pathways.

Key words: Arabidopsis, density effects, flowering time, gibberellic acid, law of constant final yield.

Environmental factors that affect plant growth include rain, wind, sunlight, and nutrition in soil. Plant density also affects plant growth. When plants are grown at high densities, the amount of nutrition from the soil decreases, and touch frequency among plants and the amount of plant hormones and growth substances that are released from plants increases (Harper 1977). Generally, plants grow smaller under high-density conditions and larger under low-density conditions (with proper spacing between plants) in what is called a "density effect" (Pacala and Weiner 1991). The stem or hypocotyl lengths, size of leaves and flowering time are often used as indicators of plant growth to investigate density effects (Akihama 1968; Jennings and de Jesus 1968; Levin and Wilson 1978).

Density effects have been reported in plants, echinoderms, protozoans, bacteria, isopoda, and insects (Fishman 1997). Competition, cooperation, and environmental factors such as light, temperature, and food determine the population density (Scrimshaw 1966). Grasshoppers grown in low-density populations live alone, but those grown in high-density populations possess better wings and live in groups. Grasshoppers grown in high-density populations often jump and move to other locations because obtaining sufficient food is difficult in the high population area. This behavior can cause locust plague and damage to crops. In this way, population density affects body weight, behavior, and oviposition of individuals, but in some species, it also affects morphology, color, and physiology (Scrimshaw 1966).

Plants under high-density conditions cannot change locations to locate nutrition, so the density effect mechanism differs. In general, plant weight under low-density conditions increases compared to high-density conditions because of the amount of available resources

Abbreviations: Arabidopsis, *Arabidopsis thaliana*; CCA1, CIRCADIAN CLOCK ASSOCIATED 1; EIN2, ETHYLENE-INSENSITIVE 2; FCA, FLOWERING CONTROL LOCUS A; FT, FLOWERING LOCUS T; ELF3, EARLY FLOWERING 3; GAI, GIBBERELLIC ACID INSENSITIVE; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYL; LL, continuous light.

[†]These authors contributed equally to this work.

This article can be found at http://www.jspcmb.jp/
Published online October 22, 2016

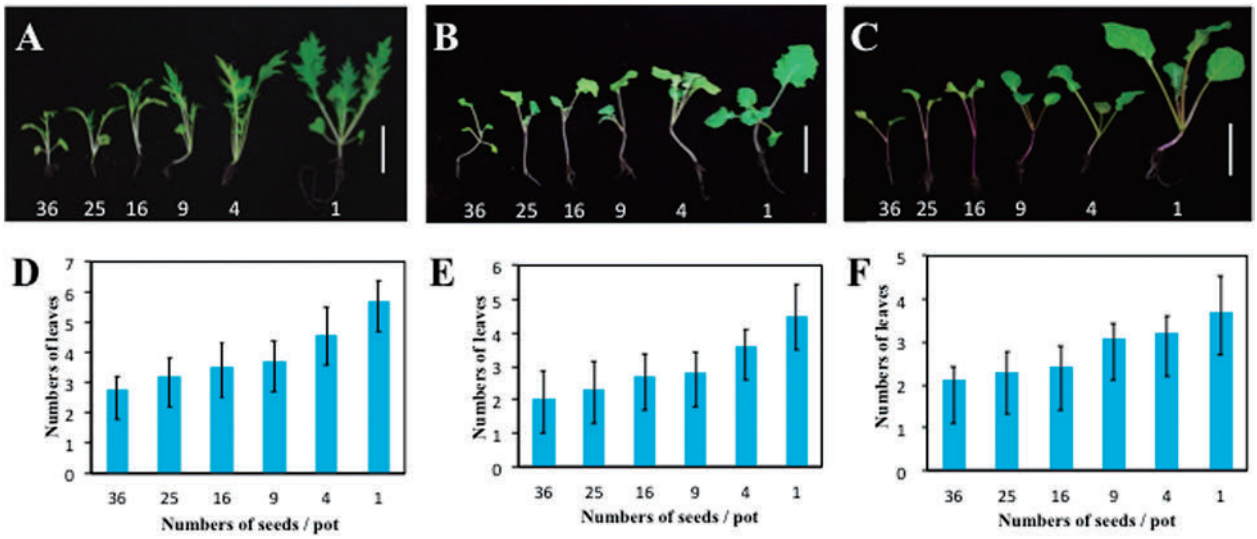

Figure 1. Density effect on Brassicaceae (*Brassica rapa* var. *nipposinica*, *Brassica napus*, *Brassica chinensis* f. *honsaitai*). (A–C) Photographs of *B. rapa* var. *nipposinica* (A), *B. napus* (B), and *Brassica chinensis* f. *honsaitai* (C). (D–F) Number of leaves of *B. rapa* var. *nipposinica* (D), *B. napus* (E), and *B. chinensis* f. *honsaitai* (F). Seeds of *B. rapa* var. *nipposinica*, *B. napus*, and *B. chinensis* f. *honsaitai* were sown (36, 25, 16, 9, 4, and 1 per pot) and plants were grown in pots (r=2.5 cm, H=5 cm) supplemented with soil (Jiffy Mix, Sakata) in controlled-environment rooms at 22°C under continuous light (LL) with a photon flux density of ~40 μmol m^{-2} s^{-1}. Plants were photographed and the numbers of leaves were counted 25 days after sowing seeds. Data are presented as means±SE (n=10). Each experiment was performed at least twice with similar results.

(Willey and Heath 1969). The yield is approximately fixed regardless of density, which is called the law of constant final yield (Kira et al. 1953; Pacala and Weiner 1991; Pearl and Parker 1922).

Ethylene is a simple gaseous plant hormone that affects numerous physiological processes in the growth and development of higher plants (Kendrick and Chang 2008). Plants release ethylene in response to environmental stresses such as being touched frequently, oxygen deficiency, wounding, and pathogen invasion. Increased ethylene levels can affect plant developmental processes such as seed germination and seedling growth, leaf abscission, organ senescence, and fruit ripening (Kendrick and Chang 2008). Many ethylene-related mutants of *Arabidopsis thaliana* (Arabidopsis) have been identified. For example, ethylene-insensitive mutants such as *ethylene responsive 1* (*etr1*; Bleecker et al. 1988) and *ethylene insensitive 2* (*ein2*; Guzmán and Ecker 1990) do not respond properly to ethylene. *constitutive triple response 1* (*ctr1*) causes constitutive ethylene response and dwarf phenotype in Arabidopsis (Kieber et al. 1993). Therefore, ethylene has been suggested to play an important role in promoting the density effects on plant growth.

Acceleration of flowering time is known to be caused by a density effect (Akihama 1968). A transition from the vegetative phase to the reproductive phase is called flowering, which is controlled by at least three distinct pathways in Arabidopsis; the long-day (LD)/photoperiod pathway, the gibberellic acid (GA) pathway, and the autonomous/vernalization pathway. The LD/photoperiod pathway is mainly affected by the circadian clock (Mizoguchi et al. 2002, 2005). In Arabidopsis, clock proteins such as LATE ELONGATED HYPOCOTYL (LHY), CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), and EARLY FLOWERING 3 (ELF3) play key roles in maintaining circadian rhythms (Alabadí et al. 2001, 2002; Mizoguchi et al. 2002). Two genes, *GIGANTEA* (*GI*) and *CONSTANS* (*CO*), promote flowering through the LD/photoperiodic pathway, while *GIBBERELLIC ACID INSENSITIVE* (*GAI*) and *FLOWERING CONTROL LOCUS A* (*FCA*) are floral activator genes of the GA and autonomous/vernalization pathways, respectively. FLOWERING LOCUS T (FT) is a floral hormone, florigen, which acts on downstream targets common to all three flowering pathways (Kardailsky et al. 1999; Kobayashi et al. 1999; Corbesier et al. 2007; Tamaki et al. 2007; Matsoukas et al. 2012; Tsuji et al. 2013). The genetic pathway *GI-CO-FT* plays a central role in the photoperiod-dependent acceleration of flowering in Arabidopsis (Mizoguchi et al. 2005).

For example, under both short-day (SD) and LD conditions, mutations in the circadian clock genes *LHY* and *CCA1* (*lhy;cca1*) accelerate flowering (Mizoguchi et al. 2002, 2005), but delay flowering under continuous light (LL; Fujiwara et al. 2008; Yoshida et al. 2009). In contrast, *elf3* flowers earlier than the wild-type (WT) under SD, LD, and LL (Yoshida et al. 2009; Zagotta et al. 1996).

To establish experimental conditions to investigate the density effects on plants, three Brassicaceae plants, namely, *B. rapa* var. *nipposinica*, *B. napus*, and *B. chinensis* f. *honsaitai* were used (Figure 1). Plant seeds at densities of 1, 4, 9, 16, 25, and 36 per pot were sown

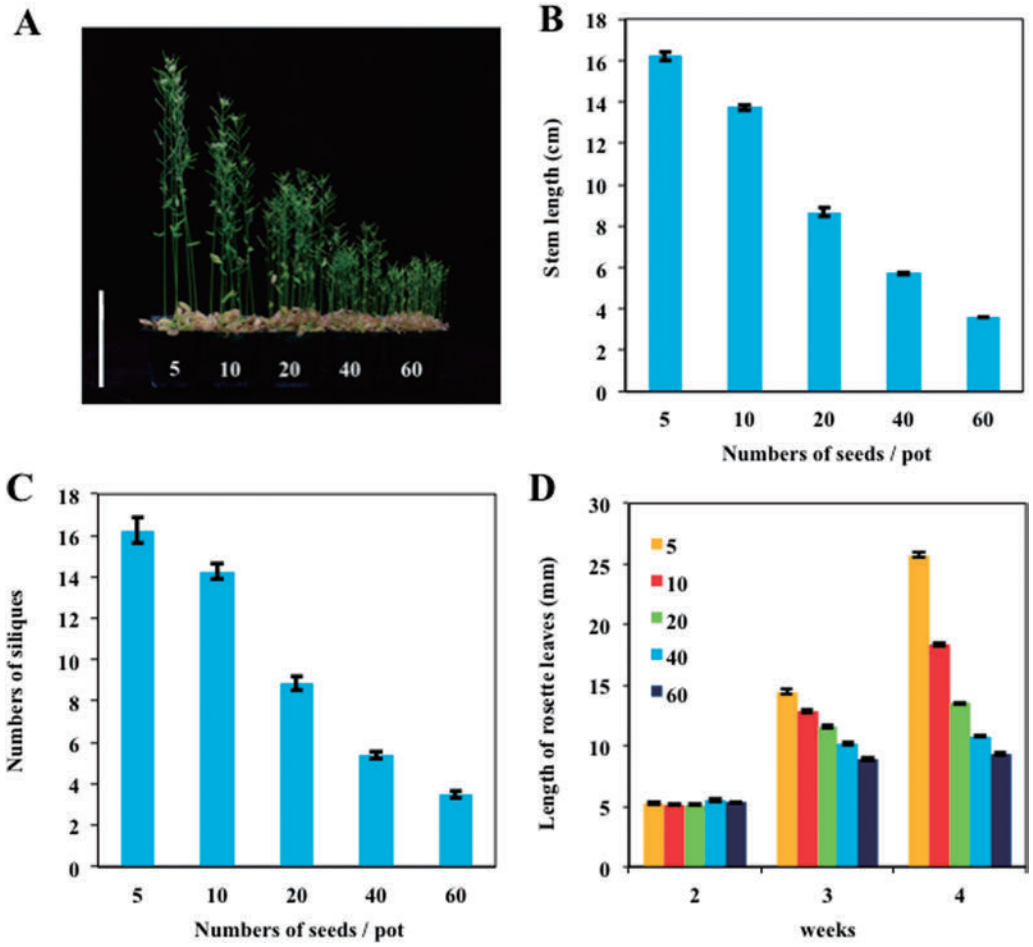

Figure 2. Density effect on stem length, number of siliques, and rosette radius of wild-type *Arabidopsis thaliana* (L*er*). (A) Photograph of plants, (B) stem lengths, (C) number of siliques, and (D) rosette radius. Seeds were imbibed and cold-treated at 4°C for 3 days in darkness before germination under light. Seeds (60, 40, 20, 10, and 5 per pot) were sown and plants were grown in pots (L=4.2 cm, D=4.2 cm, H=4.5 cm) supplemented with soil (Jiffy Mix, Sakata) in controlled-environment rooms at 22°C under continuous light (LL) with a photon flux density of ~40 μmol m^{-2} s^{-1}. Plants were photographed, stem length was measured, and the number of siliques was counted after bolting. The rosette radius (length of rosette leaves) of plants grown for 2, 3, and 4 weeks was measured. Data are presented as means±SE (n=8). Each experiment was performed at least twice with similar results.

and plants were grown for 20 days under LL. At lower densities, the number of leaves of *B. rapa* var. *nipposinica* (Figure 1A, D), *B. napus* (Figure 1B, E), and *B. chinensis* f. *honsaitai* (Figure 1C, F) increased. For example, the average numbers of leaves of *B. rapa* var. *nipposinica* at densities of 1 and 36 were 4.5 and 2, respectively. In addition, as the density decreased, the leaf size increased (Figure 1A–C).

We next confirmed the density effect on Arabidopsis under our laboratory conditions. L*er* WT seeds at densities of 5, 10, 20, 40, and 60 per pot were sown, and plants were grown for 35 days under continuous light conditions (LL). As the density decreased, the stem length and number of siliques increased (Figure 2A–C). For example, the average stem length at densities of 5 and 60 were 26.5 cm and 7.1 cm, respectively (Figure 2B). The average number of siliques of plants at densities of 5 and 60 were 9.2 and 4.2, respectively (Figure 2C).

Next, the density effect on the rosette radius of Arabidopsis was examined under the same conditions. WT (L*er*) seeds at densities of 5, 10, 20, 40, and 60 per pot were sown, and plants were grown for 1, 2, 3, and 4 weeks under LL (Figure 2D). No significant difference in rosette radius was observed at densities of 5–60 by 2 weeks, but after this time, the differences in size became significant between densities of 5–10 and 20–60. As the density decreased, the rosette radius increased with similar patterns as changes in stem lengths and the numbers of siliques. These results indicated that plant densities have significant effects on the stem length, number of siliques, and rosette radius in Arabidopsis WT (L*er*) under LL.

These results on Arabidopsis WT (L*er*), as well as *B. rapa* var. *nipposinica*, *B. napus*, and *B. chinensis* f. *honsaitai*, indicated that the laboratory conditions used in this study were appropriate to investigate the molecular mechanisms underlying density effects on plants.

To investigate the possible roles of ethylene on the density effects on flowering time, osette radius and stem

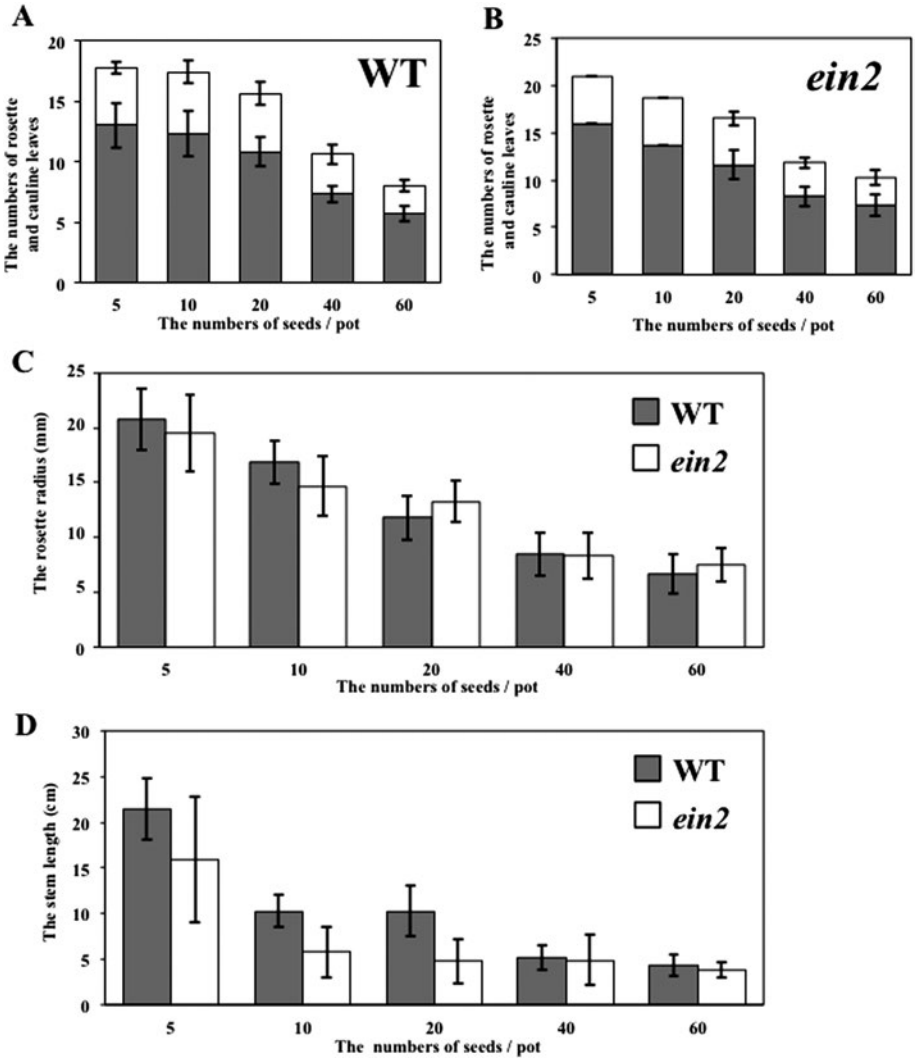

Figure 3. Density effect on stem length, rosette radius, and flowering time of an ethylene mutant of *Arabidopsis thaliana* (Col). (A, B) Flowering time of the wild type (WT; Columbia; Col) and *ethylene-insensitive 2–5* (*ein2–5*, Col, Alonso et al. 1999). Stem length (C) and rosette radius (D) of the WT (Col) and *ein2* (Col). Seeds were imbibed and cold-treated at 4°C for 3 days in darkness before germination under light. Seeds (60, 40, 20, 10, and 5 per pot) were sown and plants were grown in pots (L=2 cm, D=2 cm, H=2 cm) supplemented with soil (Jiffy Mix, Sakata) in controlled-environment rooms at 22°C under continuous light (LL) with a photon flux density of ~40 μmol m^{-2} s^{-1}. Stem length and rosette radius of plants were measured 3 weeks after sowing seeds. Flowering time was scored by counting the number of rosette and cauline leaves on the main stem after bolting. Data are presented as means±SE (n=10). Each experiment was performed at least twice with similar results.

length of Arabidopsis, WT (Col) seeds and ethylene-insensitive 2 (*ein2*) mutants were sown at densities of 5, 10, 20, 40, and 60 per pot, and plants were grown under LL (Figure 3). As the density increased, flowering time (Figure 3A), rosette radius (Figure 3C) and stem length (Figure 3D) of the WT (Col) plants decreased. *ein2* did not significantly suppress the decrease in flowering time, rosette radius and stem length (Figure 3B–D), indicating that ethylene did not play a major role in the density effects on Arabidopsis.

Although accelerated flowering time is known to be a density effect (Akihama 1968), the molecular mechanism underlying this characteristic is not fully understood. In plants, three major genetic pathways, namely, the LD, GA, and autonomous/vernalization pathways, regulate flowering time (Koornneef et al. 1991). To investigate the roles of key regulator genes of the density effect in these Arabidopsis flowering pathways, four late and one early flowering mutants were examined (Figure 4). The Arabidopsis mutants, *gi-6* (L*er*; Koornneef et al. 1991), *lhy-12;cca1–101* (L*er*; Fujiwara et al. 2008), *fca-1* (L*er*; Koornneef et al. 1991), *gai* (L*er*; Koornneef et al. 1991) and *elf3–101* (L*er*; Yoshida et al. 2009) were used in this work. Seeds of the WT (L*er*), four late flowering mutants (*gi*, *lhy;cca1*, *gai*, and *fca*), and an early flowering mutant (*elf3*) were sown at densities of 5, 10, 20, 40, and 60 per pot, and plants were grown under LL. As the density increased, the total leaf number of L*er* WT plants decreased (Figure 4A). The late flowering phenotypes of *gi*, *lhy;cca1*, and *fca* were largely suppressed as the

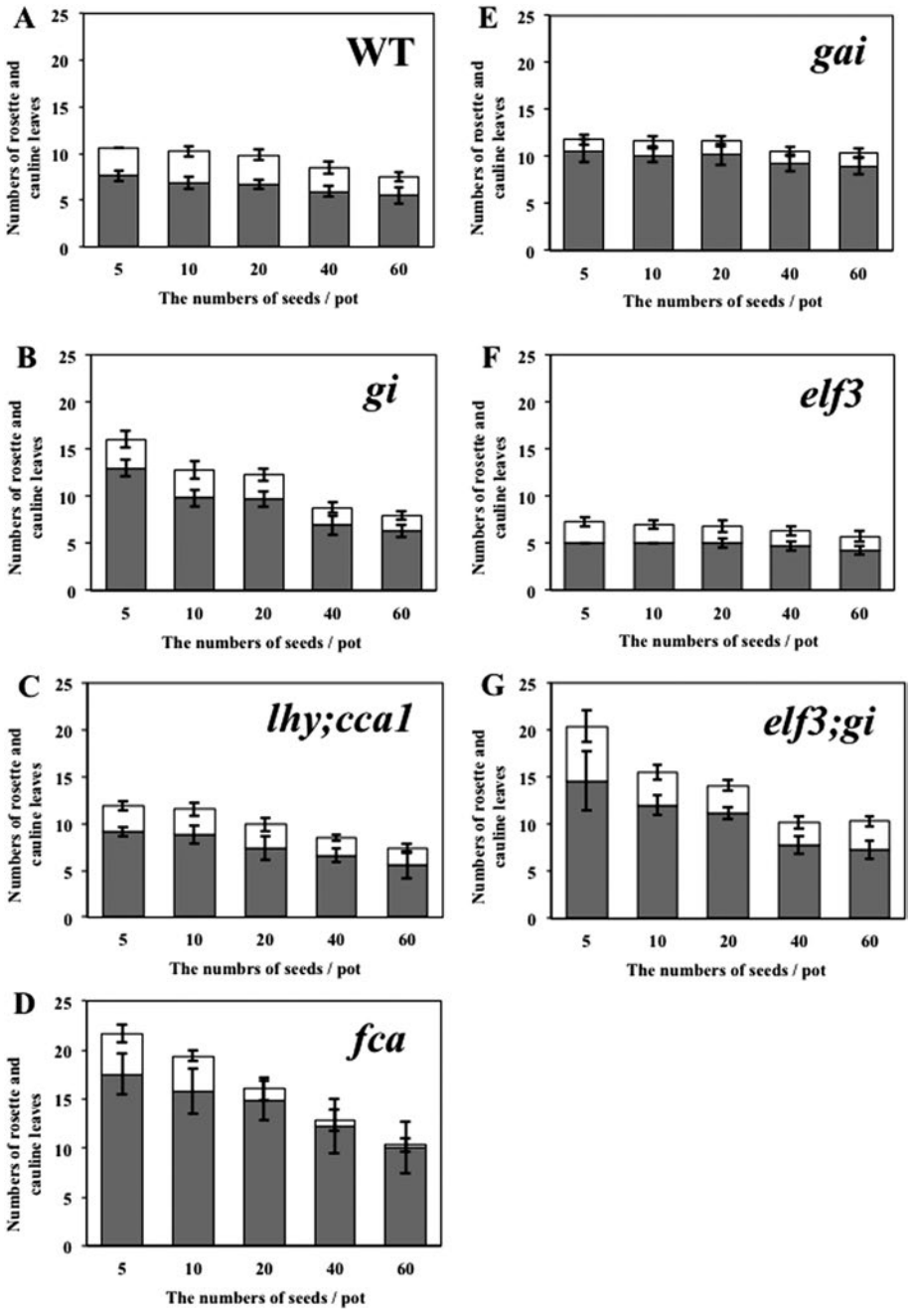

Figure 4. Density effect on flowering time of early and late flowering mutants of Arabidopsis. (A) Wild type (WT, L*er*), (B) *gi*, (C) *lhy;cca1*, (D) *fca*, (E) *gai*, (F) *elf3*, and (G) *elf3;gi*. Seeds were imbibed and cold-treated at 4°C for 3 days in darkness before germination under light. Seeds (60, 40, 20, 10, and 5 per pot) were sown and plants were grown in pots (L=2 cm, D=2 cm, H=2 cm) supplemented with soil (Jiffy Mix, Sakata) in controlled-environment rooms at 22°C under continuous light (LL) with a photon flux density of ~40 μmol m^{-2} s^{-1}. Flowering time was scored by counting the number of rosette and cauline leaves on the main stem after bolting. Data are presented as means±SE (n=10). Each experiment was performed at least twice with similar results.

density increased (Figure 4B–D). In contrast, the delay in flowering of *gai* was only slightly suppressed at high densities (Figure 4E). These results suggested that acceleration of flowering time at higher densities is dependent on the GA pathway and independent of the LD and autonomous pathways.

Acceleration of flowering at higher densities was not significantly affected by *gi* (Figure 4B). However, the early flowering phenotype of *elf3* was largely suppressed by *gi* (Figure 4F, G), suggesting that the acceleration of flowering at increased densities was not dependent on ELF3 activity. Although the accelerated flowering time of the early flowering mutant *elf3* at higher densities was only observed in 1.6 leaves, the difference was statistically significant compared to the WT (Figure 4F), suggesting that *elf3* is sensitive to the high-density. These data

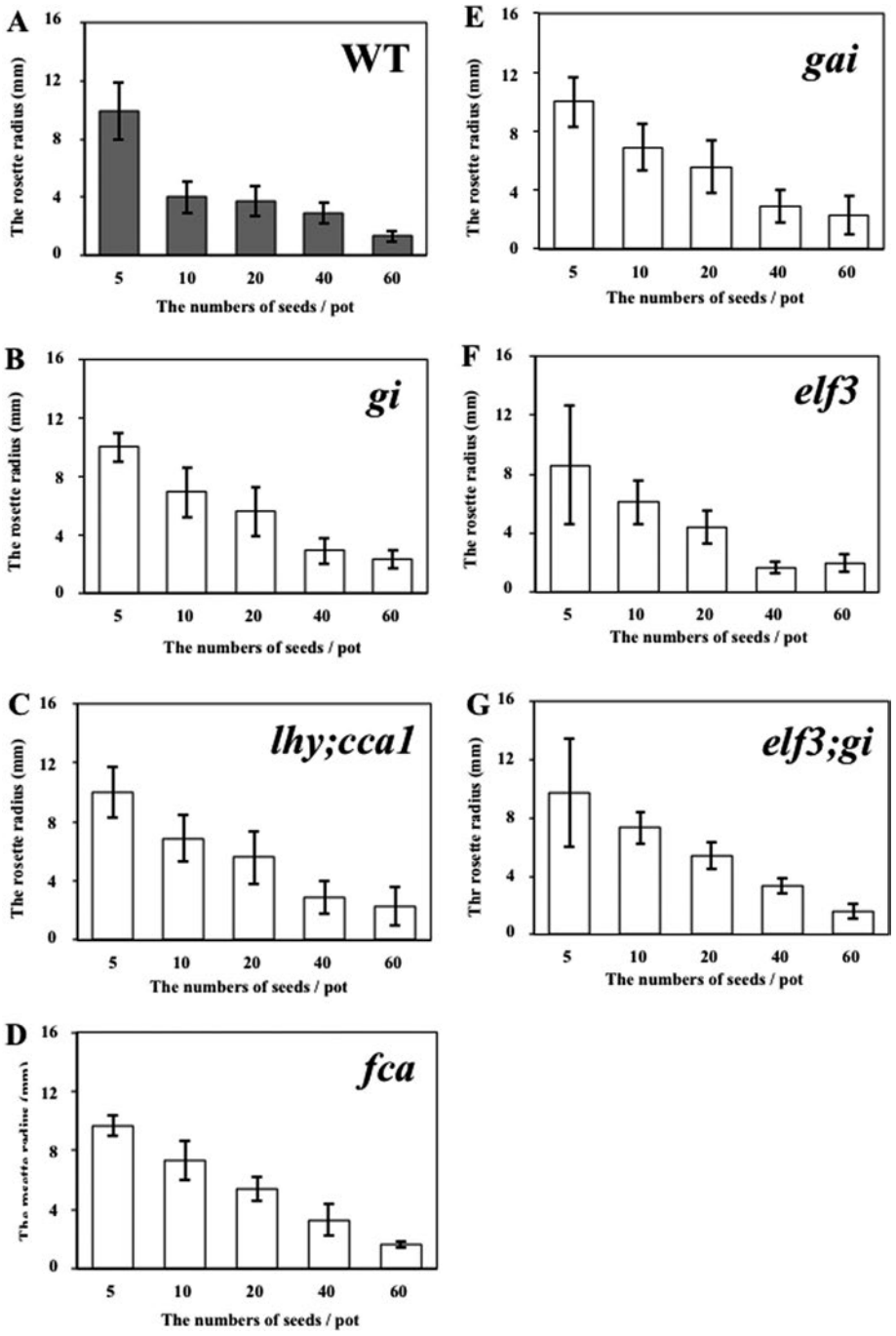

Figure 5. Density effect on rosette radius of early and late flowering mutants of Arabidopsis. (A) Wild type (WT, L*er*), (B) *gi*, (C) *lhy;cca1*, (D) *fca*, (E) *gai*, (F) *elf3*, and (G) *elf3;gi*. Seeds were imbibed and cold-treated at 4°C for 3 days in darkness before germination under light. Seeds (60, 40, 20, 10, and 5 per pot) were sown and plants were grown in pots (L=2 cm, D=2 cm, H=2 cm) supplemented with soil (Jiffy Mix, Sakata) in controlled-environment rooms at 22°C under continuous light (LL) with a photon flux density of ~40 μmol m^{-2} s^{-1}. Rosette radius of plants was measured 3 weeks after sowing seeds. Data are presented as means±SE (n=10). Each experiment was performed at least twice with similar results.

supported our hypothesis that an increased density and *elf3* promoted flowering through two distinct pathways.

Flowering time, rosette radius, and stem length were affected by higher densities. To determine whether the GA pathway plays a role in the control of rosette radius and stem length, seeds of *gai* and other controls were sown at densities of 5, 10, 20, 40, and 60 per pot and grown under LL. The rosette radius of *gai* decreased at higher densities, similar to the WT (Figure 5A, E). Other mutations such as *gi*, *lhy;cca1*, *fca*, *elf3*, and *elf3;gi* (Natsui et al. 2010) did not significantly affect the decrease in rosette radius at higher densities (Figure 5). These results indicated that GAI, ELF3, GI, LHY, CCA1, and FCA do not play an important role in the decrease in rosette radius at higher densities.

If GA plays an important role in the shortening of

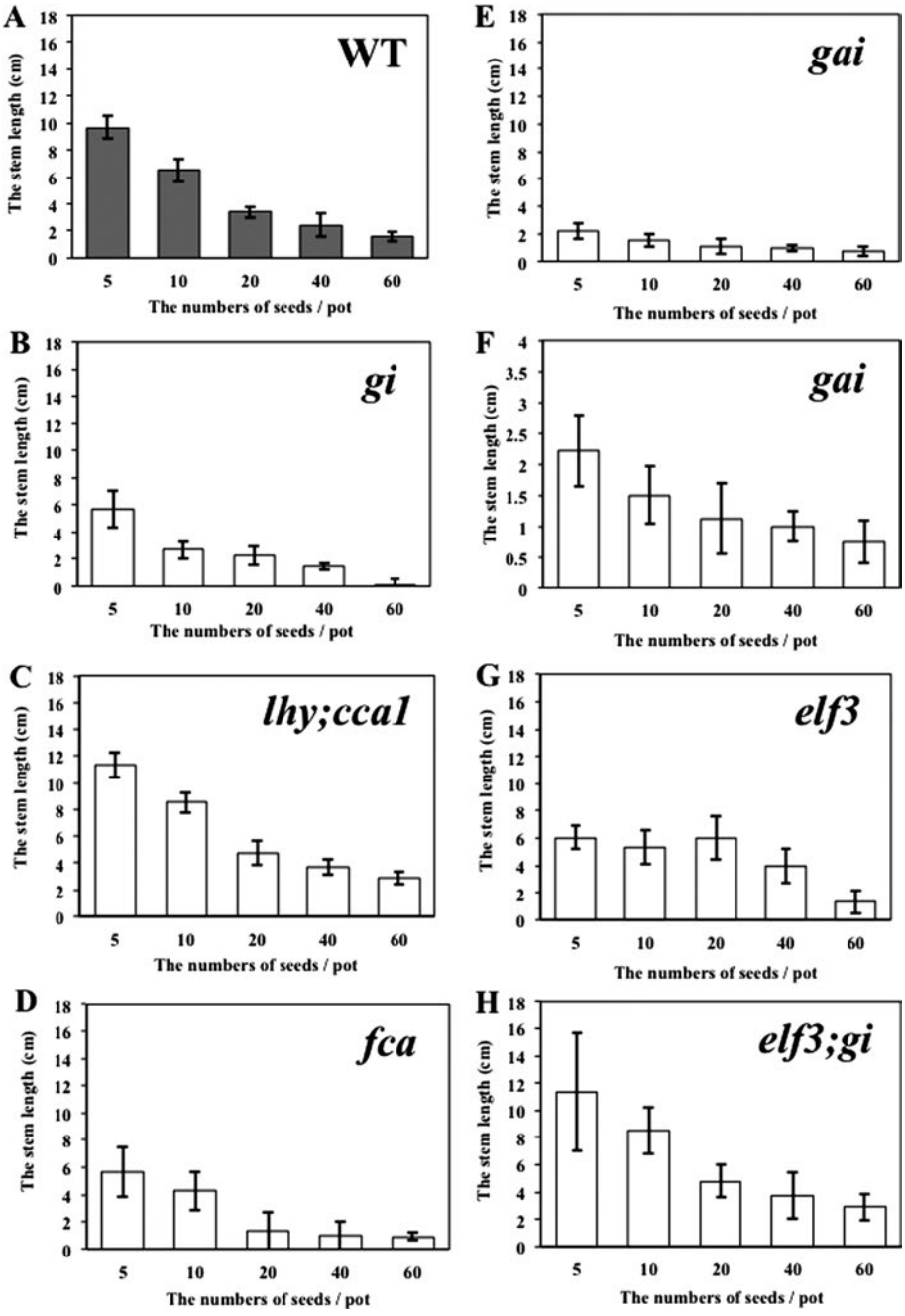

Figure 6. Density effect on stem length of early and late flowering mutants of Arabidopsis. (A) Wild type (WT, L*er*), (B) *gi*, (C) *lhy;cca1*, (D) *fca*, (E, F) *gai*, (G) *elf3*, and (H) *elf3;gi*. (F) is an enlarged image of (E). Seeds were imbibed and cold-treated at 4°C for 3 days in darkness before germination under light. Seeds (60, 40, 20, 10, and 5 per pot) were sown and plants were grown in pots (L=2 cm, D=2 cm, H=2 cm) supplemented with soil (Jiffy Mix, Sakata) in controlled-environment rooms at 22°C under continuous light (LL) with a photon flux density of ~40 μmol m^{-2} s^{-1}. Data are presented as means±SE (n=10). Each experiment was performed at least twice with similar results.

stem length at higher densities, this process should be suppressed by *gai*. However, stem length of *gai* at a density of 5 was significantly reduced compared to the WT (Figure 6A, E, F). Reduction of stem length in *gai* still occurred at densities of 60 (Figure 6E, F), suggesting that GA did not play an important role in high-density-dependent shortening of stem length. Loss of function of GI, LHY, CCA1, and FCA did not significantly affect the reduction of stem length at higher densities (Figure 6).

As shown in Figures 1–6, our results in the Arabidopsis WT (L*er* and Col) and Brassicaceae (*B. napus*, *B. rapa* var. *nipposinica*, *B. chinensis* f. *honsaitai*) plants indicated that plants grow smaller under high-density conditions and larger under low-density conditions (with proper spacing between plants). This is known as the "law of constant final yield" (Pacala and Weiner 1991).

Ethylene is thought to play an important role in

density effects. Therefore, we expected that ethylene-insensitive mutants (*etr1*, *ein2*, *ein3*, and *ein5*; Bleecker et al. 1988; Guzmán and Ecker 1990) would grow larger than the WT at high densities. However, *ein2* and the WT showed no significant difference in rosette radius, stem length, or flowering time (Figure 3), indicating that ethylene does not play a major role in density effects under our experimental conditions. However, a detailed analysis of other ethylene-related mutants such as *etr1*, *ein3*, *ein5*, *ctr1*, *eto1*, *eto2*, and *eto3* (Bleecker et al. 1988; Guzmán and Ecker 1990) is required. Based on these results, unidentified factors other than ethylene may play a role in the density effects in plants.

We proposed that the accelerated flowering time could be used to investigate density effects. Regulation of flowering time has been well studied because it is stable and easy to investigate. Many genes that control flowering time have been identified in model plants such as Arabidopsis (Song et al. 2013), *Oryza sativa* (Tsuji et al. 2011), and *Pisum sativa* (Weller et al. 2009).

At high densities, the Arabidopsis WT (L*er* and Col) flowered earlier than at low densities. The acceleration of flowering time at high densities is not likely due to shade avoidance alone. Although the acceleration of flowering time at higher densities was not affected by *gi* and *lhy;cca1*, the early flowering phenotype of *phyB* mutants was partly suppressed by a mutation in the *CO* gene, which is a downstream factor of GI, LHY, and CCA1 in the LD pathway (Mizoguchi et al. 2005; Putterill et al. 1995; Reed et al. 1993). In contrast, *gai* appeared less-sensitive to density effects, suggesting that the acceleration of flowering at high densities may require key components of the GA pathway. Whether other mutations in the biosynthesis and signaling of GA affect the acceleration of flowering time at high densities remains unknown.

Florigen is a plant hormone that accelerates flowering time (Matsoukas et al. 2012; Tsuji et al. 2013). *FT* encodes florigen, and many genes in the three major flowering pathways positively and negatively affect the gene expression of *FT* (Corbesier et al. 2007; Kardailsky et al. 1999; Kobayashi et al. 1999; Tamaki et al. 2007). Expression of the *FT* gene is likely increased in plants grown at high densities. *FT*, as well as *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and *LEAFY* (*LFY*), are known to be floral integrators (Komeda 2004). Expression of these three genes is increased by GA (Mutasa-Göttgens and Hedden 2009). Detailed analysis on the expression of the floral integrator and regulator genes would increase our understanding of the molecular mechanism underlying density effects.

Although GA appears to play a positive role in the high-density-dependent acceleration of flowering, GA is unlikely to have a major role in the control of stem length and rosette radius at high densities (Figures 5 and 6). Regulators of flowering time such as GI, LHY, CCA1, and FCA are also unlikely to be involved in these processes (Figures 5 and 6).

Identification of key steps in the biosynthesis or signaling of GA for acceleration of flowering at high densities is an important challenge. Thus, whether GA-insensitive or -deficient mutants in other plant species show similar phenotypes as Arabidopsis *gai* should be explored. Genetic screening of Arabidopsis mutants in which stem length, rosette radius, or flowering time are not affected by high densities is important to understand the molecular mechanisms underlying the law of constant final yield.

Acknowledgements

This study was supported in part by the joint research programs "Plant Transgenic Research Design, University of Tsukuba" of the MEXT (to T.M.), the joint research program implemented at the Institute of Plant Science and Resources, Okayama University, of the MEXT (to T.M.), Grants-in-Aid for Scientific Research on Priority Areas (MEXT; Grant number 23012037 to T.M.) and grants-in-aid for Scientific Research (C) (MEXT; Grant number 25440141 to T.M.).

References

Akihama T (1968) Competitive ability in mutant lines of a rice variety. *Japan J Breed* 18: 72–74

Alabadí D, Oyama T, Yanovsky MJ, Harmon FG, Más P, Kay SA (2001) Reciprocal regulation between *TOC1* and *LHY*/*CCA1* within the *Arabidopsis* circadian clock. *Science* 293: 880–883

Alabadí D, Yanovsky MJ, Más P, Harmer SL, Kay SA (2002) Critical role for CCA1 and LHY in maintaining circadian rhythmicity in *Arabidopsis*. *Curr Biol* 12: 757–761

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science* 284: 2148–2152

Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* 241: 1086–1089

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, et al. (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 316: 1030–1033

Fishman MA (1997) Density effects in population growth: An exploration. *Biosystems* 40: 219–236

Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G, et al. (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. *Plant Cell* 20: 2960–2971

Guzmán P, Ecker JR (1990) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. *Plant Cell* 2: 513–523

Harper JL (1977) Population biology of plant. Academic Press.

Jennings PR, de Jesus J (1968) Studies on competition in rice I. competition in mixtures of varieties. *Evolution* 22: 119–124

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation

tagging of the floral inducer FT. *Science* 286: 1962–1965
Kendrick MD, Chang C (2008) Ethylene signaling: New levels of complexity and regulation. *Curr Opin Plant Biol* 11: 479–485
Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) *CTR1*, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. *Cell* 72: 427–441
Kira T, Ogawa H, Shinozaki K (1953) Intraspecific competition among higher plants. I: Competition-yield-density interrelationships in regularly dispersed populations. *J Inst Polytech Osaka City Univ* D4: 1–16
Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 286: 1960–1962
Komeda Y (2004) Genetic regulation of time to flower in *Arabidopsis thaliana*. *Annu Rev Plant Biol* 55: 521–535
Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol Gen Genet* 229: 57–66
Levin DA, Wilson JB (1978) The genetic implication of ecological adaptation in plants. In: Freysen AHJ, Woldendrop JW (eds) *Structure and function of plant populations*, North Holland Publ Co, Amsterdam, pp 75–100
Matsoukas IG, Massiah AJ, Thomas B (2012) Florigenic and antflorigenic signaling in plants. *Plant Cell Physiol* 53: 1827–1842
Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, Carré IA, Coupland G (2002) *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in *Arabidopsis*. *Dev Cell* 2: 629–641
Mizoguchi T, Wright L, Fujiwara S, Cremer F, Lee K, Onouchi H, Mouradov A, Fowler S, Kamada H, Putterill J, et al. (2005) Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in Arabidopsis. *Plant Cell* 17: 2255–2270
Mutasa-Göttgens E, Hedden P (2009) Gibberellin as a factor in floral regulatory networks. *J Exp Bot* 60: 1979–1989
Natsui Y, Nefissi R, Miyata K, Oda A, Hase Y, Nakagawa M, Mizoguchi T (2010) Isolation and characterization of suppressors of the *early flowering 3* in *Arabidopsis thaliana*. *Plant Biotechnol* 27: 463–468
Pacala SW, Weiner J (1991) Effects of competitive asymmetry on a local density model of plant interference. *J Theor Biol* 149: 165–179
Pearl R, Parker SL (1922) On the influence of density of population upon the rate of reproduction in *Drosophila*. *Proc Natl Acad Sci USA* 8: 212–219
Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80: 847–857
Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. *Plant Cell* 5: 147–157
Scrimshaw NS (1966) Application of nutritional and food science to meeting world food needs. *Proc Natl Acad Sci USA* 56: 352–359
Song YH, Ito S, Imaizumi T (2013) Flowering time regulation: photoperiod- and temperature-sensing in leaves. *Trends Plant Sci* 18: 575–583
Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. *Science* 316: 1033–1036
Tsuji H, Taoka K, Shimamoto K (2011) Regulation of flowering in rice: Two florigen genes, a complex gene network, and natural variation. *Curr Opin Plant Biol* 14: 45–52
Tsuji H, Taoka K, Shimamoto K (2013) Florigen in rice: Complex gene network for florigen transcription, florigen activation complex, and multiple functions. *Curr Opin Plant Biol* 16: 228–235
Weller JL, Hecht V, Liew LC, Sussmilch FC, Wenden B, Knowles CL, Vander Schoor JK (2009) Update on the genetic control of flowering in garden pea. *J Exp Bot* 60: 2493–2499
Willey RW, Heath SB (1969) The quantitative relationships between plant population and crop yield. *Adv Agron* 21: 281–321
Yoshida R, Fekih R, Fujiwara S, Oda A, Miyata K, Tomozoe Y, Nakagawa M, Niinuma K, Hayashi K, Ezura H, et al. (2009) Possible role of EARLY FLOWERING 3 (ELF3) in clock-dependent floral regulation by SHORT VEGETATIVE PHASE (SVP) in *Arabidopsis thaliana*. *New Phytol* 182: 838–850
Zagotta MT, Hicks KA, Jacobs CI, Young JC, Hangarter RP, Meeks-Wagner DR (1996) The *Arabidopsis ELF3* gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. *Plant J* 10: 691–702