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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：　Genetic improvement and protected cultivation of low-chill peach
Title of Dissertation　（少低温要求性モモの遺伝的改良と施設栽培）

学位論文要約：
Dissertation Summary

The future chilling accumulation patterns were projected to decline both quantity and duration due to global warming. Insufficient winter chilling accumulation can detrimentally impact temperate fruit productions (Parker and Abatzoglou, 2019). Hence, the countermeasures, such as the low-chill cultivars, must be developed to prevent or mitigate the adverse effects of elevated temperatures and insufficient chilling accumulation. One of the long-term solutions for adapting and mitigating global warming threats is breeding new peach cultivars with low chilling requirements. Low-chill cultivars have high adaptability to warmer environments in subtropical regions or tropical highlands. In addition, these cultivars may be adapted to temperate climates, where the absence of frost occurs in spring (Topp et al., 2008). The low chilling requirement of these cultivars means that they flower in mid- to late winter, leading to harvest earlier than high chill peach cultivar. From this advantage, low-chill cultivars might be used to produce extreme early harvest season with higher fruit price by introducing to forcing culture. Therefore, this research focused on breeding new low-chill peach cultivars and illustrated the growing a low-chill peach cultivar in forcing culture.

1. Genetic improvement, embryo rescue, and seedling evaluation for new low-chill peach

New peach cultivars that adapt well to mild winter regions and deliver high fruit quality and good yields are crucial to sustaining peach production under climate change situations. Cruz et al. (2007) projected increases in average surface temperatures over Japan, it would increase the risk for insufficient chill accumulation in temperature fruit trees. Low-chill variety might become more extensively grown if global warming trends continue. Although low-chill cultivars are well adapted to warm conditions, they can be grown in a temperate zone where is in the absence of spring frost that destroys the flower and young leaf (Topp et al., 2008). Thereby, cultivars with low chilling requirements also provide more sustainable cultivars for future temperate fruit production in Japan. Under these conditions, the demand for low-chill varieties would rise noticeably.

The low-chill peach breeding program by Kagawa University, Japan, was initiated in 2016. The main objective of the breeding program is to develop new low-chill peach cultivars with early ripening and fruit quality traits greater or similar to our low-chill peaches ‘KU-PP1’ and ‘KU-PP2’. Two of our low-chill peach cultivars and two advanced selections were used as parents to transfer the preferred traits to their progenies. Twelve crosses were created by combining parents (‘KU-PP1’, ‘KU-PP2’, HFP1, and Flordaglo x Hikawa Hakuho) in both open pollination and various combination, leading to a greater quantity of hybrid seedlings with superior characteristics in the next generation. Due to the high fruit prices for the early market, the low-chill genotypes are required short FDP with early fruit maturation to ensure that the fruits are harvested in the extreme early season. Thus, a short FDP is one of the major objectives in our low-chill peach breeding programs to generate low-chill peach cultivars with early ripening. Consequently, breeders must use both early ripening genotypes as male and female parents for producing hybrid seedlings with ultra-early ripening. Thereby, seed from genotypes with a short fruit development period (FDP; less than 90 days) that is counted immature and obliged to germinate. Due to rapid flesh softening and quick fruit ripening, the embryos of this peach genotype do not have sufficient time to achieve full physiological maturity, resulting in embryo abortion. Aseptic embryo culture in a nutrient-rich environment can overcome embryo abortion and enhance embryo germinability of early ripening genotype. The appropriate embryo development stage is crucial to prevent embryo abortion resulting in better embryo germinability and seedling survival. In the same way, medium and culture conditions must be suitable for the embryo development stage and for each genotype. The very small embryos require the more specialized medium and culture techniques. For this reason, this study aimed to determine correct stage of embryo growth, suitable medium, and proper culture techniques for recovering germination and improve hybrid seedling growth and survival. Then, hybrid seedling's characteristics were also evaluated in the field. The promising low chill with early maturating seedlings were selected.

Our study showed that the germination of the immature embryo was influenced by three factors: surface sterilization, culture technique with fruit age and culture conditions. The whole fruit sterilizing with 1.2% of sodium hypochlorite with 0.05% of Tween 20 for 15 minutes was the most effective treatment to use in the initial sterilization. The ovule culture with modified SH agar-gelled medium + 0.1% activated charcoal was more suitable for the embryo of 'KU-PP1' that harvest from advanced maturity while embryo rescue with WPM medium + vermiculite was useful for culturing 'KU-PP2' embryo at fruit ripening stage. Moreover, we found that the LED light can stimulate seedling growth in terms of shoot and root length.

Under natural conditions in Kagawa, we found that nineteen selected hybrids adapted and grew very well in Kagawa prefecture, which is the mild winter region. Floral buds of most selected hybrid seedlings emerged in mid-January to mid-February. The budburst of all selected seedlings began earlier than their parents and 'Hikawa Hakuho', a commercial cultivar but they bloomed together with some parents or earlier than 'KU-PP1' and 'Hikawa Hakuho' (Table 1). In the other words, these distinct hybrids have lower chilling requirement than the parents and 'Hikawa Hakuho'. Melting flesh types in both white and yellow flesh were selected. Fruit must have high sugar content, medium size with a round to flat shape and absence of split pits (Table 2 and Table 3). Selected genotypes are planted in a 56L container to evaluate growth performance and fruit productivity prior to testing on a commercial scale. The fruit of our selected peaches is round with a flattened tip; 8 in 19 seedlings are yellow-fleshed genotypes (Table 2 and Table 3). After the second-year evaluation, we selected four hybrid peaches (17003 T5, 17004 T17, 17004 T18, and 17004 T21) as the advanced selections. 17004 T18 are better in firmness when compared with standard cultivars and other selections. There is greater durability for managing and transporting to distant markets. All these advanced selections showed outstanding characteristics in both years (2020 and 2021), and there is potential to be new cultivar candidates for lower chilling requirements, earlier ripening, or high anthocyanin peaches. These selections will be evaluated actual chilling requirements by excised shoots technique under controlled conditions in winter 2021.

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Table 1 Parents and performance of selected seedlings, parents, and a commercial cultivar in Kagawa (Japan) from 2020 to 2021.

Genotypes	Parent	Budburst date	Full bloom date	First ripe date	FDP (days)	Floral bud density	Severity of leaf curl disease
Commercial cultivar							
'Hikawa Hakuho'		30 Mar (89[z])	7 Apr (97)	10 Jul (191)	94	Medium	Resistant
Parents							
'KU-PP1'		20 Feb (51)	13 Mar (72)	08 Jun (159)	87	Very dense	Moderately susceptible
'KU-PP2'		13 Feb (44)	12 Mar (71)	10 Jun (161)	90	Very dense	Moderately susceptible
HFP1		20 Feb (51)	08 Mar (67)	10 Jun (161)	94	Very dense	Moderately susceptible
Flordaglo × Hikawa Hakuho		15 Jan (15)	03 Mar (62)	12 Jun (163)	101	Medium	Moderately susceptible
Selected genotypes							
17003 T3	HFP1 OP	01 Feb (32)	12 Mar (71)	14 Jun (165)	94	Very dense	Average resistant
17003 T5	HFP1 OP	01 Feb (32)	12 Mar (71)	08 Jun (159)	88	Very dense	Average resistant
17003 T6	HFP1 OP	01 Feb (32)	12 Mar (71)	08 Jun (159)	88	Very dense	Average resistant
17004 T5	KU-PP1 OP	21 Jan (21)	06 Mar (65)	03 Jun (154)	89	Medium	Average resistant
17004 T14	KU-PP1 OP	15 Jan (15)	06 Mar (65)	31 May (151)	86	Medium	Average resistant
17004 T17	KU-PP1 OP	24 Feb (55)	18 Mar (77)	17 Jun (168)	91	Very dense	Average resistant
17004 T18	KU-PP1 OP	27 Jan (27)	06 Mar (65)	17 Jun (168)	103	Very dense	Average resistant
17004 T21	KU-PP1 OP	12 Feb (43)	12 Mar (71)	03 Jun (154)	83	Very dense	Average resistant
17005 T4	KU-PP1× KU-PP2	12 Feb (43)	12 Mar (71)	08 Jun (159)	88	Medium	Average resistant
17005 T8	KU-PP1× KU-PP2	09 Feb (40)	12 Mar (71)	31 May (151)	80	Very dense	Average resistant
17006 T5	KU-PP2 OP	09 Jan (09)	06 Mar (65)	31 May (151)	86	Very dense	Average resistant
18003 T15	HFP1 OP	09 Feb (40)	09 Mar (68)	21 Jun (172)	104	Medium	Average resistant
18003 T31	HFP1 OP	17 Jan (17)	09 Mar (68)	09 Jun (160)	92	Medium	Average resistant
18003 T35	HFP1 OP	24 Jan (24)	12 Mar (71)	31 May (151)	80	Medium	Average resistant
18004 T4	KU-PP1 OP	21 Jan (21)	12 Mar (71)	25 Jun (176)	105	Very dense	Average resistant
18004 T11	KU-PP1 OP	14 Jan (14)	03 Mar (62)	17 Jun (168)	106	Very dense	Average resistant
18005 T15	KU-PP1× KU-PP2	01 Feb (32)	15 Mar (74)	09 Jun (160)	86	Very dense	Average resistant
18005 T17	KU-PP1× KU-PP2	27 Jan (27)	12 Mar (71)	21 Jun (172)	101	Medium	Average resistant
18005 T20	KU-PP1× KU-PP2	21 Jan (21)	06 Mar (65)	09 Jun (160)	95	Medium	Average resistant

[z] Values in parentheses indicate the Julian date.

[Y] Fruit development period (FDP) was computed as the number of days from flower blooming to fruit harvest.

Table 2 Fruit qualities of selected seedlings, parents, and a commercial cultivar in Kagawa (Japan) from 2020 to 2021.

Genotypes	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)		TSS (°Brix)	Flesh firmness (N)	Flesh type	Flesh-to-stone adherence	Pit-splitting
			Suture	Cheek					
Commercial cultivar									
‘Hikawa Hakuho’	180.39	6.94	6.71	7.10	11.9	0.46	Melting	Clingstone	None
Parents									
‘KU-PP1’	112.07	5.35	5.86	6.29	12.3	0.57	Melting	Clingstone	20%
‘KU-PP2’	131.31	5.60	6.32	6.36	13.8	0.30	Melting	Clingstone	80%
HFP1	165.78	5.71	6.98	7.14	11.6	0.35	Melting	Clingstone	20%
Flordaglo × Hikawa Hakuho	183.27	6.48	6.80	7.34	10.1	0.31	Melting	Clingstone	100%
Selected genotypes									
17003 T3	107.77	5.30	5.09	6.05	11.7	0.34	Melting	Semi-freestone	40%
17003 T5	90.53	4.99	5.45	5.59	12.7	0.32	Melting	Semi-freestone	None
17003 T6	68.32	4.80	4.75	5.26	10.8	0.39	Melting	Freestone	40%
17004 T5	55.24	4.08	4.68	4.92	14.1	0.35	Melting	Clingstone	20%
17004 T14	38.98	3.71	4.00	4.27	12.6	0.51	Melting	Clingstone	20%
17004 T17	81.51	4.72	5.25	5.73	10.2	0.55	Melting	Semi-freestone	None
17004 T18	65.62	4.24	4.89	5.33	19.0	0.82	Melting	Semi-freestone	None
17004 T21	67.03	4.86	4.62	5.13	14.6	0.36	Melting	Semi-freestone	None
17005 T4	111.42	5.94	5.93	6.08	14.3	0.27	Melting	Semi-freestone	None
17005 T8	58.04	4.59	4.58	4.83	12.2	0.40	Melting	Clingstone	40%
17006 T5	87.97	5.39	5.12	5.77	12.2	0.41	Melting	Clingstone	100%
18003 T15	83.65	4.56	5.48	5.73	16.1	0.50	Melting	Clingstone	40%
18003 T31	69.34	4.42	5.10	5.17	15.3	0.44	Melting	Clingstone	20%
18003 T35	64.00	4.53	4.87	5.00	16.3	0.40	Melting	Clingstone	None
18004 T4	98.26	5.36	5.60	5.56	15.4	0.68	Melting	Freestone	50%
18004 T11	58.68	4.26	4.68	4.87	17.6	0.52	Melting	Clingstone	None
18005 T15	44.04	4.14	4.41	4.55	17.4	0.35	Melting	Clingstone	None
18005 T17	64.44	4.60	4.84	5.00	14.2	0.33	Melting	Freestone	None
18005 T20	58.39	4.07	4.68	5.07	21.7	0.39	Melting	Clingstone	None

Table 3 Fruit appearance of selected seedlings, parents, and a commercial cultivar in Kagawa (Japan) from 2020 to 2021.

Genotypes	Flesh colour	Fruit shape	Tip	Ground colour	Over colour	Extent over colour	Pattern cover skin colour
Commercial cultivar							
‘Hikawa Hakuho’	Cream white	Ovate	No tip	Greenish-white	Pink red	30–45%	Solid
Parents							
‘KU-PP1’	Snow white	Round	No tip	Pink white	Medium red	75–90%	Striped
‘KU-PP2’	Yelllow	Round	No tip	Light yellow	Light red	15–30%	Solid
HFP1	Snow white	Oblate	No tip	Cream-white	Medium red	75–90%	Striped
Flordaglo × Hikawa Hakuho	Snow white	Round	No tip	Cream-green	Pink red	75–90%	Solid
Selected genotypes							
17003 T3	Yellow	Round	No tip	Orange yellow	Dark red	90–100%	Marbled
17003 T5	Yellow	Round	No tip	Orange yellow	Light red	30–45%	Solid
17003 T6	Orange yellow	Oblate	No tip	Orange yellow	Medium red	30–45%	Marbled
17004 T5	Cream white	Oblate	No tip	Cream green	Medium red	45–60%	Marbled
17004 T14	Greenish-white	Oblate	No tip	Greenish-white	Medium red	75–90%	Marbled
17004 T17	Snow white	Oblate	No tip	Greenish-yellow	Medium red	60–75%	Striped
17004 T18	Yellow	Oblate	No tip	Orange yellow	Medium red	60–75%	Solid
17004 T21	Greenish-white	Round	No tip	Cream green	Medium red	30–45%	Solid
17005 T4	Cream white	Round	No tip	Cream white	Medium red	30–45%	Striped
17005 T8	Snow white	Round	No tip	Greenish-yellow	Medium red	30–45%	Marbled
17006 T5	Yellow	Oblate	No tip	Greenish-yellow	Medium red	10–15%	Solid
18003 T15	Orange yellow	Oblate	No tip	Orange yellow	Dark red	75–90%	Striped
18003 T31	Orange yellow	Oblate	No tip	Orange yellow	Dark red	75–90%	Solid
18003 T35	Snow white	Round	No tip	Cream white	Dark red	60–75%	Marbled
18004 T4	Greenish-white	Round	No tip	Greenish-yellow	Medium red	30–45%	Mottled
18004 T11	Light yellow	Round	No tip	Orange yellow	Blackish-red	90–100%	Solid
18005 T15	Cream white	Round	No tip	Cream white	Medium red	30–45%	Solid
18005 T17	Cream white	Round	No tip	Greenish-yellow	Dark red	75–90%	Solid
18005 T20	Snow white	Oblate	No tip	Cream white	Medium red	30–45%	Marbled

2. Optimization of growth temperature for growing 'KU-PP2', a low-chill peach in protected cultivation

Under forcing conditions, spring phenology, fruit development, and plant productivity are mainly driven by temperature. The appropriate temperature for each plant development stage could enhance plant growth, maximize the yield potential, avoid unnecessary energy usage, and keep cost-effective for forcing culture system. Additionally, the air temperature manipulation during dormancy releasing to harvesting can hasten fruit maturation. Dela Bruna (2007), who investigated the fruit development of peaches in subtropical regions, reported that elevated temperatures accelerated progress from budburst to fruit maturation due to a shorter time in initial phases of fruit development. Temperature influences not only the time of fruit maturity but also the fruit growth rate (Adams et al., 2001). Warrington et al. (1999) found the positive relationship between temperature and rate of fruit expansion in apple. The apple fruit expansion rate increased to approximately ten times when the temperature had been raised from 6°C to 20°C.

Even though the optimum temperatures stimulate spring phenological events, enhance fruit development, and accelerate fruit ripening, excessive-high temperatures might have the opposite effect to plant development and fruit production through retarded the growth of reproductive organs or disrupting normal plant functions such as carbon assimilation, respiration, fertilization, and cell differentiation (Cui et al., 2006; Efeoglu and Terzioglu, 2009; Lin-Wang, 2011; Hao et al., 2019). However, the effect of high temperature on plants differs depending on the duration of exposure and the stages of plant development. During budburst and flowering, the temperature at 20°C and above suppressed the embryo sac development, diminish the pollen viability and interrupt fertilization; consequently, the fruit set rate is poor (Erez, 2000; Kozai et. al., 2004; Hatfield et al., 2011). Hasanuzzaman et. al. (2013) indicated that chlorophyll contents and the chlorophyll a/b ratio in soybeans grown under high temperature (38/28°C) decreased approximately 5% and 18%, respectively. As well, continuing high-temperature exposure can cause slowing of plant growth and inducing an imbalance in carbohydrate metabolism between photosynthesis and respiration, resulting in insufficient carbohydrate supply to subsidize plant development, leading to yield loss, and possibly plant death (Hall, 1992; Wahid et al., 2007). In apple (*Malus domestica*), the elevated temperature was associated with a reduction in phytochemical components and softening of fruit flesh (Sugiura et. al., 2003).

This study aimed to demonstrate the cultivation of low-chill peach cultivars under forcing conditions for producing extreme early season. Obviously, plant development and fruit productivity under forcing conditions are directly and indirectly affected by atmospheric temperatures around the plants. The temperature in the greenhouse or plastic house is regulated to maximize fruit production. However, the plant response to the ambient temperature depends on their cultivars and plant development stage. Knowledge of the relationship between forcing temperatures and the growth performance at each development stage of plants is the key to success in growing them under forcing conditions and is useful to establish suitable management practices for maximum productivity. Thereby, three experiments were conducted in this study to clarify the effects of temperature on morpho-anatomy and physiology of the low-chill peach under controlled temperature conditions and to compare the ability of the plastic house with and without a heating system for hastening peach harvest season. 'KU-PP2', a low-chill peach cultivar, and the plastic house with heating and ventilation systems were used in this investigation as a model plant and forcing culture archetype, respectively. A better understanding of the low-chill peach response to growth temperature could be utilized to design a forcing program and cultural practices management to amplify the yield potential and optimize profitability.

During budburst and flowering, our results indicated that prolonged chilling exposure and higher forcing temperatures hastened bud burst and flowering (Fig. 1 and Fig. 2), as well as increased the level and uniformity of bud break in the 'KU-PP2' peach cultivar. However, inadequate chilling exposure and excessive forcing temperatures negatively affected dormancy-breaking, flower development, anthesis, and fruit set in 'KU-PP2'.

Budburst and flowering were significantly retarded by insufficient chilling and elevated forcing temperatures of 20–25°C used in this study. During fruit development, the high-temperature conditions enhanced fruit growth in early fruit development stages (S1 and S2; Table 5), hastened the harvesting period (Table 5), and stimulated red coloration of the fruit peel (Fig. 5); however, such conditions decreased leaf size and thickness (Table 4 and Fig. 3), chlorophyll contents (Fig. 4), carbon assimilation rate. Moreover, high temperature retarded late fruit development (S3 stage; Table 5) and negatively affected fruit quality.

Finally, the growing of a low-chill peach in plastic houses with and without a heating system were demonstrated. The plastic houses used for this purpose were 6 m wide, 12 m long, and 3.5 m high with an area of 72 m^2. The results show that the forcing conditions accelerated the spring phenology and harvest period of ‘KU-PP2’. The bud bursting of ‘KU-PP2’ in the plastic house with a heating system occurred in mid-February, resulting in the acceleration blooming (late February) and fruit harvesting (mid-May), which were earlier than blooming and fruit harvest under natural conditions by 4 and 6 weeks, respectively. In the same way, the plastic house without a heating system hastened blooming, and harvesting by up to 3 and 4 weeks, respectively when compared with the open field conditions. Although the fruit growth pattern did not differ significantly between the forcing and natural conditions, the rate of fruit growth at stages S1 and S2 was higher in the plastic house with a heating system than that under the other assessed conditions. Additionally, the fruit size and quality of the trees in the plastic house were higher than those under open field condition. The forcing culture has been approved to be effective in preventing pit-splitting.

Table 4 Leaf morphological and anatomical traits, percentage of leaf dry matter (DM), and stomatal density of the 'KU-PP2' peach cultivar throughout two consecutive year (2020 and 2021). The peach trees were grown under three growing temperature regimes (20°C, 25°C, and 30°C).

Parameters	Growth temperature			*p*-value
	20°C	25°C	30°C	
Leaf length (cm)	16.9 ± 0.3 a [z]	17.3 ± 0.5 a	12.9 ± 0.7 b	< 0.0001
Leaf width (cm)	4.7 ± 0.3 a	4.9 ± 0.1 a	3.5 ± 0.5 b	0.0153
Percentage of leaf dry matter (% FW)	41.3 ± 0.98 b	42.9 ± 0.77 b	52.1 ± 0.54 a	< 0.0001
Stomatal density (no. mm $^{-2}$)	239 ± 9 b	267 ± 8 a	164 ± 11 c	< 0.0001
Leaf thickness (μm)	60 ± 2.1 a	43 ± 1.7 b	36 ± 3.5 c	< 0.0001
Adaxial epidermis thickness (μm)	6 ± 0.4	5 ± 0.3	6 ± 0.3	0.4571
Abaxial epidermis thickness (μm)	4 ± 0.2	3 ± 0.4	4 ± 0.3	0.7468
Palisade mesophyll thickness (μm)	27 ± 0.9 a	20 ± 1.2 b	14 ± 1.7 c	< 0.0001
Spongy mesophyll thickness (μm)	23 ± 0.4 a	15 ± 0.7 b	12 ± 0.6 c	0.0007

[z] Data are the average values ± standard errors (n = 20). The different lower-case letters within the same row denote significant differences at $p \leq 0.05$ (Tukey's HSD test)

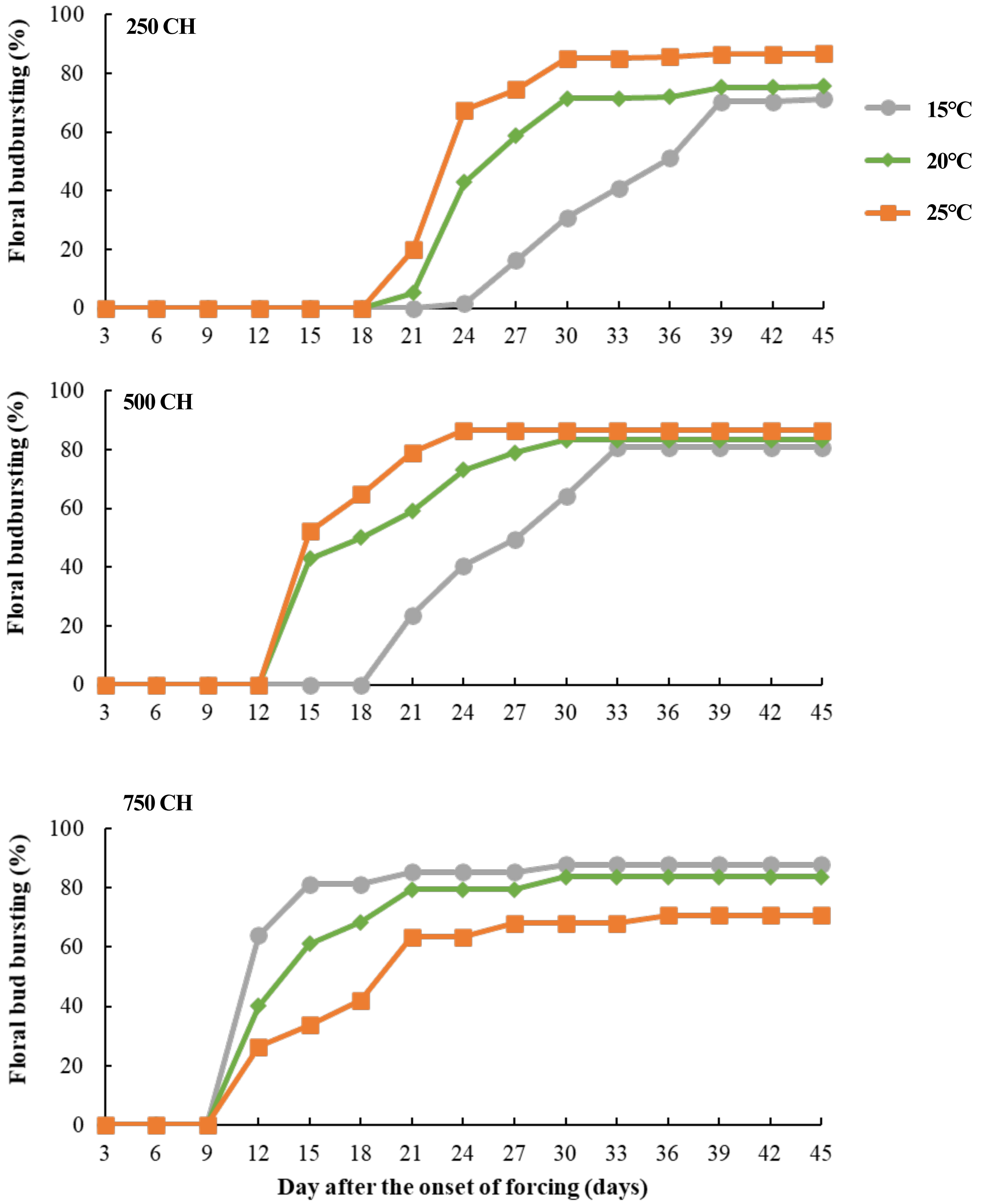

Figure 1 The average accumulative of floral budburst (%) of 'KU-PP2' peach trees throughout the experiment in two consecutive seasons after chilling accumulation reached 250, 500, and 750 CH. And then the trees were treated to the different forcing temperature levels (15, 20, and 25°C). Budburst was observed every three days after the onset of forcing conditions.

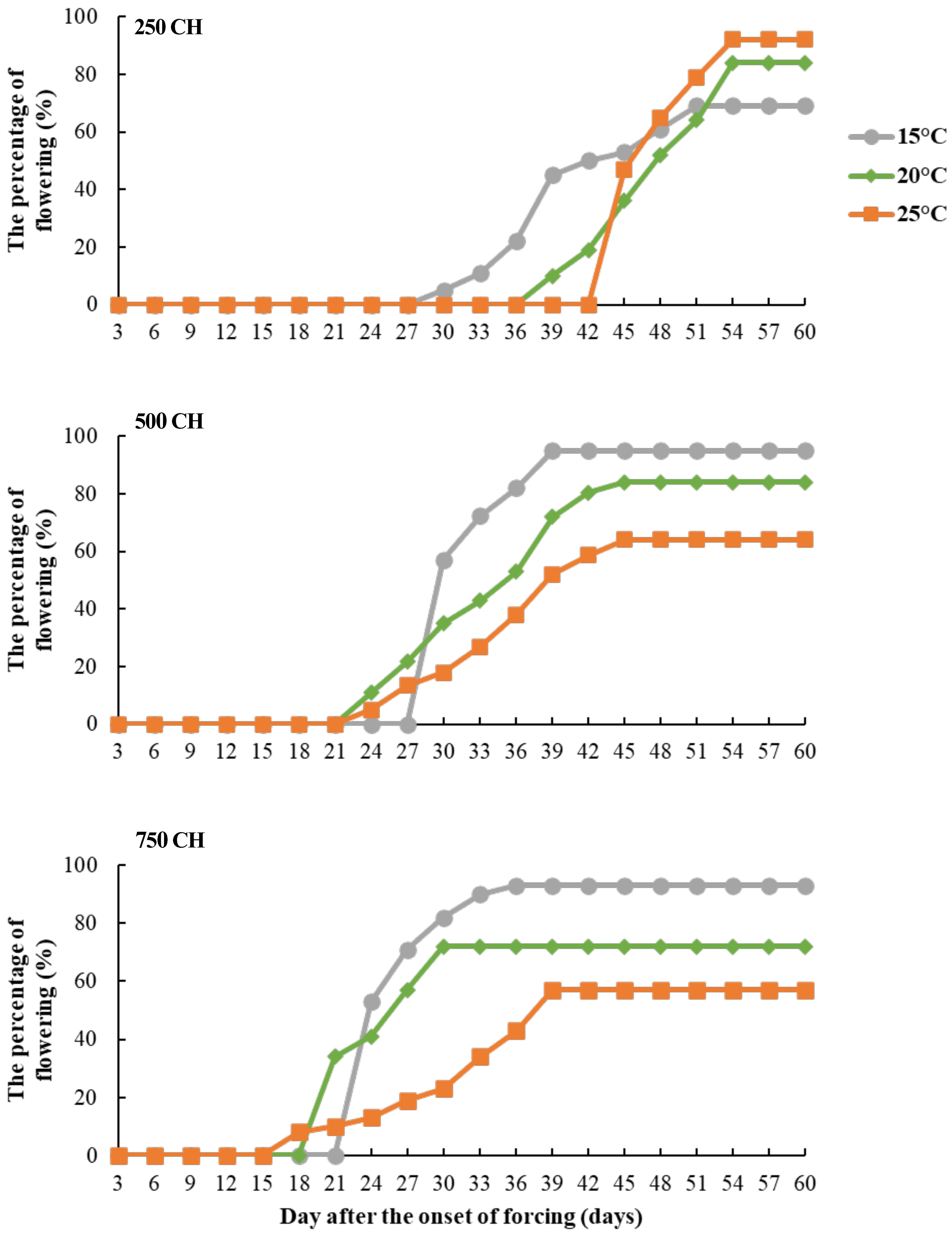

Figure 2 The average accumulative of flower opening (%) of ‘KU-PP2’ peach trees throughout the experiment in two consecutive seasons after chilling accumulation reached 250, 500, and 750 CH. And then the trees were treated to the different forcing temperature levels (15, 20, and 25°C). Budburst was observed every three days after the onset of forcing conditions..

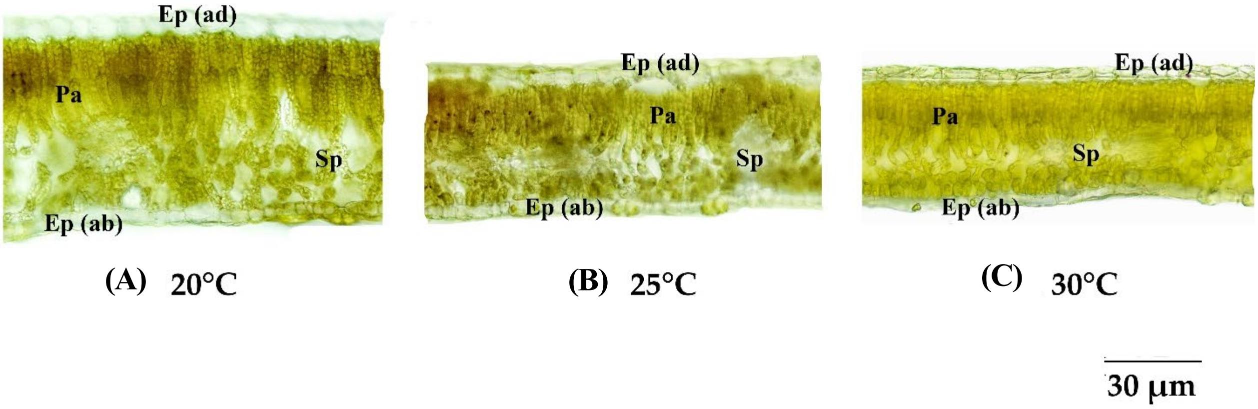

Figure 3 The microscopic images of leaf cross-section of 'KU-PP2' peach trees that were grown under (A) 20°C, (B) 25°C, and (C) 30°C. The cross-sections of leaf sample were studied and photographed by a light microscope at the end of experiment. Ep (ad) = adaxial epidermis; Pa = Palisade mesophyll layer; Sp = Spongy mesophyll layer Ep (ab) = abaxial epidermis. These pictures were taken on 15 November 2020.

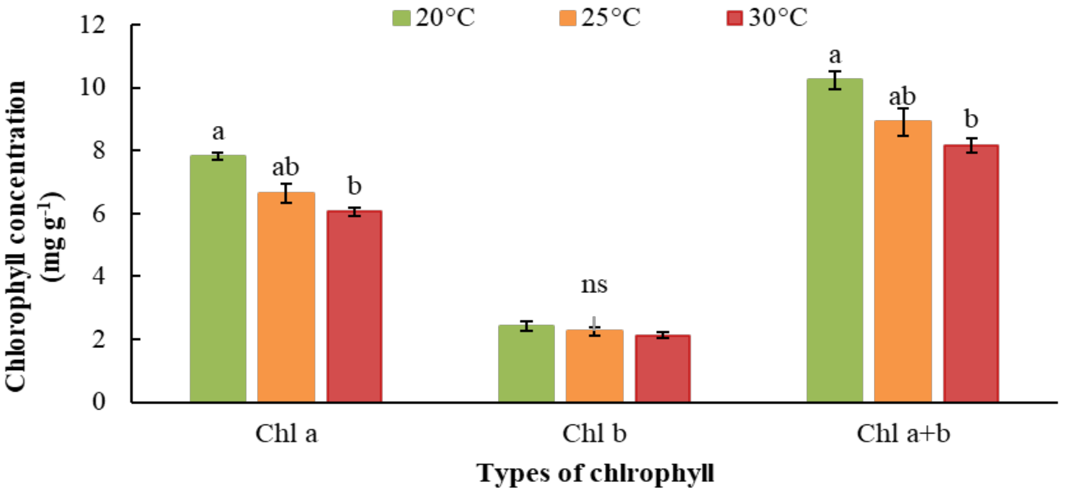

Figure 4 Chlorophyll content in response to growth temperature treatments. Data expressed as means ± standard error (n = 10). Different letters denote significant differences according to Tukey's test ($p \leq 0.05$) and ns indicates non-significant.

Table 5 Fruit diameter expansion rate, harvest date, and fruit development period of the 'KU-PP2' fruits for each temperature regime in two consecutive years (2020 and 2021).

Temperature regime	Development stage duration (d)				Harvest date	
	S1	S2	S3	Total	2020	2021
20°C	36 a [z]	30 a	30 b	96 a	9 Jun (161) [y]	21 June (172)
25°C	30 b	24 b	36 a	90 b	31 May (152)	14 June (165)
30°C	24 c	18 c	36 a	78 c	24 May (145)	4 June (155)
p–value	0.0003	< 0.0001	0.0063	0.002	–	–

Data was log transformed before statistical analysis.

[z] Data are expressed as mean values ± standard errors (n = 20). Different lowercase letters within the same row denote significant differences ($p \leq 0.05$; Tukey's test).

[y] Harvest date was defined as the date of first harvest. Values in parentheses indicate Julian calendar harvest dates.

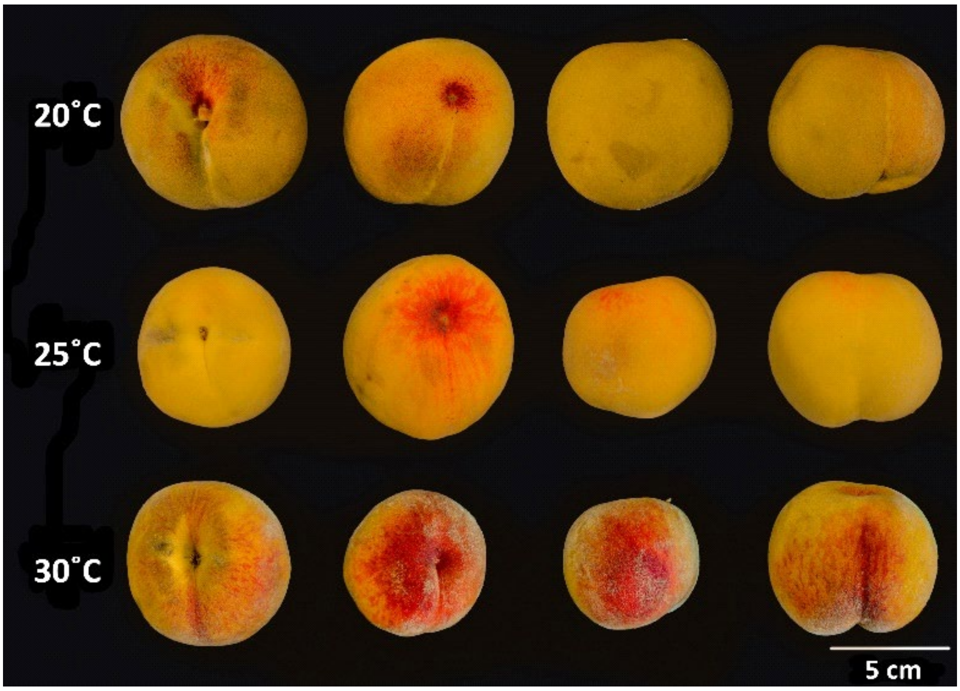

Figure 5 Effect of growth temperature on the colouration of the 'KU-PP2' fruits during the commercial ripening period.

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