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The Differentiation of Chilling Requirements of Kiwifruit Cultivars Related to Ploidy Variation

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Abstract. **Kiwifruit (*Actinidia chinensis* Planchon) is an economically important fruit, and its flowering and production are affected by the chill accumulation in winter. In this study, the chilling requirements of nine kiwifruit cultivars with three ploidy levels (diploid, tetraploid, and hexaploid) were analyzed by using the Dynamic Model, Utah Model, and chilling hours (CH) Model. The chilling requirements for vegetative budbreak of these kiwifruit cultivars were 24–55 chill portions (CP), 316–991 chill units (CU), and 222–853 CH, and the chilling requirements for floral emergence were 45–69 CP, 825–1336 CU, and 655–1138 CH. The chilling requirements for vegetative budbreak and floral emergence were significantly lower for diploid than hexaploid cultivars with tetraploid cultivars intermediate. Pearson correlation analysis indicated that ploidy levels were positively correlated with chilling requirement, with the CV of 0.74 and 0.82 for vegetative budbreak and floral emergence chilling requirements, respectively. In conclusion, these results provide some novel insights of kiwifruit varieties of various chilling requirements, which is beneficial for kiwifruit cultivar selection for different climates and environments.**

Dormancy is an adaptation of perennial plants to cease growth in response to extreme environmental conditions (Rohde and Bhalerao, 2007). Dormancy is induced by diverse factors in different plants (Vegis, 1964), whereas dormancy termination is usually influenced by chilling in winter (Fuchigami et al., 1982; Perry, 1971; Wareing, 1956). Temperature and duration of chilling are key components of satisfying chilling requirements which are critical to promote vegetative growth and budbreak for perennial plants in the subsequent year (Perry, 1971). Temperatures near 5 °C are required to satisfy the chilling requirement of perennial plants (Perry, 1971). However, the duration requirement of chilling varies considerably between and within species (Alburquerque et al., 2008; Brundell, 1975; Egea et al., 2003; Ruiz et al., 2007; Wall et al., 2008). The Richardson's CU is often used to determine chilling requirement (Richardson et al., 1974). For example, the chilling requirements ranged from 266 to 996 CU among almond cultivars (Egea et al., 2003) and ranged from 800 to 1200 CU among apricot cultivars (Ruiz et al., 2007). Negative effects that are caused by insufficient or exceeded chill accumulation could result in reduction of budbreak or earlier flowering, which threaten the cultivation and commercialization of the perennial crop. Thus, finding the chilling requirements of plants is vital for the basic understanding of dormancy and practical application of dormancy release or bloom delay techniques (Naor et al., 2003).

Kiwifruit (*Actinidia*) is a functionally dioecious liana with variable ploidy levels (e.g., diploid, tetraploid, and hexaploid) (Huang et al., 2000; Li et al., 2010). Most current kiwifruit cultivars are selections of natural *Actinidia chinensis* and *Actinidia deliciosa* from China (Ferguson and Huang, 2007). Compared with other fruit trees, limited information is available about the effects of temperature on the chilling requirement in the *Actinidia* genus. It has been reported that *Actinidia rufa* displayed a low-chill trait (200 h) followed by *A. chinensis* (400–600 h), and *A. deliciosa* cultivars showed the highest chilling requirements (600–800 h) (Kulthinee et al., 2004). In addition, there is an obvious variation of chilling requirements among various cultivars of an *Actinidia* species. For example, 'Hayward' (*A. deliciosa*) had a chilling requirement of more than 950 CU for vegetative buds and 1150 CU for floral buds, whereas 'Bruno' (*A. deliciosa*) may have lower chilling requirements (Caldwell, 1989). However, information has not been reported about the chilling requirements of other kiwifruit cultivars, especially for the new released cultivars with diverse ploidy levels.

Because of China's fluctuating climate and diverse environment (Luedeling, 2012), the appropriate chilling requirements of kiwifruit cultivars are essential for cultivar selection and cultivation. The objectives of this study were to 1) determine chilling requirements for vegetative budbreak and floral emergence of different kiwifruit cultivars and 2) investigate the relationship of chilling requirements with different ploidy levels.

Materials and Methods

Plant material. The plant material used in this study comprised nine kiwifruit cultivars with three ploidy levels including the diploids 'Jinyu' (originate from Henan province), 'Donghong' (Henan), and 'Wuzhi-7' (Jiangxi); the tetraploids 'Jintao' (Jiangxi), 'Jinxia' (Jiangxi), and 'H-15' (Hunan); and the hexaploids 'Jinkui' (Hubei), 'Chuanmi-1' (Sichuan), and 'Bruno' (New Zealand). The ploidies of these cultivars have been determined by flow cytometry experiments (Huang, 2014). The former five cultivars belong to *Actinidia chinensis*, and the remaining cultivars belong to *Actinidia deliciosa*. All these kiwifruit cultivars were cultivated at the National Actinidia Germplasm Repository (NAGR) at the Wuhan Botanical Garden, Chinese Academy of Science.

Experimental design. The experiments were conducted in 2014 and 2015 in the NAGR orchard. Hourly temperatures were collected from September to March by an electronic thermohygrograph (Zoglab Model DSR-TH; Zoglab, Hangzhou, China). The starting date for chilling accumulation was considered when "positive chill-units begin to accumulate just after the day in the autumn when the largest negative accumulation is experienced" (Erez et al., 1979; Richardson et al., 1974). For the Utah Model, the largest negative accumulation of CU was detected on 2 Nov. (data not shown), hence we began calculating CU on 3 Nov. For the Dynamic Model and CH Model, we began calculating CP and CH on 1 Sept., respectively (Zhang

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Table 1. Chill accumulation under field conditions in Wuhan, China (2014–15), according to the dynamic model, Utah model, and chilling hours (CH) model.

Model	1 Nov.	3 Nov.	15 Nov.	30 Nov.	15 Dec.	31 Dec.	15 Jan.	31 Jan.	15 Feb.	28 Feb.
Portions (dynamic model)	0	1	6	13	24	35	44	55	61	67
Chill units (Utah model)	—	10.5	70.0	193.5	408.5	603.5	784.5	963.5	1,049.0	1,143.5
CH (CH model)	0	7	23	45	242	436	614	828	965	965

and Taylor, 2011). For each kiwifruit cultivar, 30 canes (lengths: 30–40 cm, diameter: 5 mm) with about five buds were collected on 15 Dec., 29 Dec., 6 Jan., 23 Jan., and 8 Feb., respectively. These 30 canes were then evenly spaced in a greenhouse in triplicate at 22 °C with natural sunlight and 65% relative humidity and maintained for several weeks. Shoot counts that emerged from buds were recorded daily. Floral buds were also counted, as well as the stage of development of each flower, including full bloom, petal fall, and senescence. Once a bud developed into a shoot with flowers, it was regarded as a floral bud. Data were collected until leaves appeared chlorotic or necrotic and floral parts stopped developing or had abscised (Wall et al., 2008).

Determination of chilling requirements. The percentages of vegetative budbreak and floral emergence were calculated out of total buds and were used as indicators of chilling requirement. The chilling requirement for each kiwifruit cultivar was estimated using regression analysis which was performed based on the percentages of vegetative budbreak and floral emergence and the chilling accumulation. The optimal regression equation with the highest regression coefficient was selected for further analysis. The percentages of vegetative budbreak and floral emergence reaching 50% indicate the fulfillment of chill requirement for budbreak and flower, respectively (Zhang and Taylor, 2011). Thus, the chill requirements for vegetative budbreak and floral emergence were estimated to be the CP (Dynamic Model), CU (Utah Model), and CH (CH Model) when the percentages of vegetative budbreak and floral emergence were equal to 50%, respectively.

Statistical analysis. Statistical analysis was performed using SPSS® Statistics V21 (Chicago, IL). All data are reported as mean values with standard deviations. Differences among diploid, tetraploid, and hexaploid were tested by using one-factor analysis of variance (ANOVA). When ANOVA was significant, the means were discriminated using the Tukey's test for the post hoc pairwise comparisons. The correlation between chilling requirement and ploidy levels was estimated by the use of the Pearson correlation coefficient.

Table 2. The percentages of vegetative budbreak and floral emergence for canes with different chilling accumulations in nine kiwifruit cultivars.

		Vegetative budbreak (%)					Floral emergence (%)				
Cultivars	Ploidy	24[z]	33	38	48	59	24	33	38	48	59
Jinyu	2*x*	16 a	43 b	54 ce	71 be	62 ab	7 b	24 e	41 e	44 b	55 b
Donghong	2*x*	54 e	57 e	62 e	86 e	90 e	29 d	30 f	38 e	57 c	69 c
Wuzhi-7	2*x*	34 d	37 ab	43 b	70 b	69 b	5 b	19 d	21 c	38 ab	51 ab
Jintao	4*x*	16 a	26 a	38 ab	52 a	54 a	8 b	21 de	28 d	44 b	45 ab
Jinxia	4*x*	22 ab	29 a	47 bc	63 b	64 ab	15 c	23 de	26 cd	43 b	54 b
H-15	4*x*	29 bcd	32 a	40 ab	41 a	55 a	8 b	20 de	22 cd	33 ab	48 ab
Jinkui	6*x*	16 a	27 a	42 ab	48 a	50 a	0 a	0 a	3 a	36 ab	37 a
Chunmi-1	6*x*	25 bc	29 a	32 a	50 a	57 ab	7 b	10 b	12 b	27 a	43 ab
Bruno	6*x*	31 cd	34 ab	37 ab	48 a	63 ab	5 b	15 c	20 c	31 ab	48 ab

Means followed by different letters in the same column differ significantly at 5% of the Tukey's multiple range tests.
[z]The chill accumulation for treated canes in the field conditions according to the dynamic model.

Table 3. Chilling requirements for vegetative budbreak and floral emergence in nine kiwifruit cultivars.

	Chilling requirements for vegetative budbreak[z]			Chilling requirements for floral emergence		
Cultivars	Portions	CU	CH	Portions	CU	CH
Jinyu	42 b	693 ab	572 b	53 ac	961 ab	820 ab
Donghong	24 a	316 a	222 a	45 a	825 a	655 a
Wuzhi-7	40 b	673 ab	543 b	59 ad	1,086 ab	908 ab
Jintao	52 bc	962 b	776 bc	60 bcd	1,110 ab	1,013 b
Jinxia	44 bc	728 b	617 bc	58 ab	1,027 ab	925 ab
H-15	55 c	991 b	832 c	63 bcd	1,179 ab	961 b
Jinkui	54 c	961 b	853 c	67 bcd	1,262 b	1,041 b
Chunmi-1	52 bc	913 b	769 bc	69 bd	1,336 b	1,138 b
Bruno	49 bc	956 b	746 bc	63 bcd	1,202 b	956 ab

Means followed by different letters in the same column differ significantly at 5% of the Tukey's multiple range tests.
[z]The chilling requirements represent the chilling accumulation for 50% vegetative budbreak and floral emergence.
CH = chilling hours; CU = chill units.

Table 4. Association analysis of ploidy levels with chilling requirements in kiwifruit.

	Chilling requirements for vegetative budbreak[z]			Chilling requirements for floral emergence		
Ploidy level	Portions	CU	CH	Portions	CU	CH
Diploid	35 ± 9 a	560 ± 212 a	446 ± 194 a	52 ± 7 a	957 ± 130 a	794 ± 128 a
Tetraploid	50 ± 5 ab	894 ± 144 ab	742 ± 111 ab	60 ± 2 ab	1,105 ± 76 ab	966 ± 44 ab
Hexaploid	52 ± 2 b	943 ± 26 b	789 ± 56 b	66 ± 3 b	1,267 ± 67 b	1,045 ± 91 b

Means followed by different letters in the same column differ significantly at 5% of the Tukey's multiple range tests.
[z]The chilling requirements represent the chilling accumulation for 50% vegetative budbreak and floral emergence.
CH = chilling hours; CU = chill units.

Results

Chilling accumulation under field conditions. The chilling accumulation between 1 Sept. and 28 Feb. was measured by 'CP', 'CU', and 'CH' (Table 1). The results of the three models all revealed that positive chilling accumulation began on 3 Nov. December and January were the two most efficient months in relation to chill accumulation. In all, more than 60 CP, 1000 CU, and 900 CH of chill accumulation were detected until 28 Feb. in Wuhan, which was cold enough to study the chilling requirement of kiwifruit.

Chilling requirements for vegetative budbreak and floral emergence. The chilling accumulations until 15 Dec., 29 Dec., 6 Jan., 23 Jan., and 8 Feb. were calculated to be 24, 33, 38, 48, and 59 CP for the Dynamic Model; 271, 581.5, 679, 869, and 1025 CU for the Utah Model; and 242, 406, 500, 711, and 927 CH for the CH Model, respectively. In most cases, the percentages of vegetativebudbreak and floral emergence gradually increased for the canes with increased chilling accumulations (Table 2). The chill requirements representing the chill accumulations for 50% vegetative budbreak and floral emergence were shown in Table 3. The chilling requirements for vegetative budbreak and floral emergence of the nine kiwifruit cultivars were 24–55 CP (316–991 CU or 222–853 CH) and 45–69 CP (825–1336 CU or 655–1138 CH), with the lowest values in 'Donghong'. At the chilling accumulation of 24 CP (271 CU or 242 CH), the percentages of vegetative budbreak and floral emergence in 'Donghong' were significantly higher than that of other cultivars (Fig. 1). Within the tetraploid or hexaploid cultivars, there was no significant difference of chill requirement between any pairs of cultivars. However, within diploid cultivars, 'Donghong' showed significantly lower chilling requirement for budbreak than 'Jinyu' and 'Wuzhi-7'. For all cultivars, the chilling requirements for vegetative budbreak were lower than that for floral emergence.

Correlation between chilling requirements and ploidy levels. The average values of chilling requirements for vegetative budbreak and floral emergence in diploid cultivars were 35 and 52 CP (560 and 957 CU or 446 and 794 CH), respectively, which were both significantly lower than those in hexaploid cultivars (Table 4). The average correlation coefficients

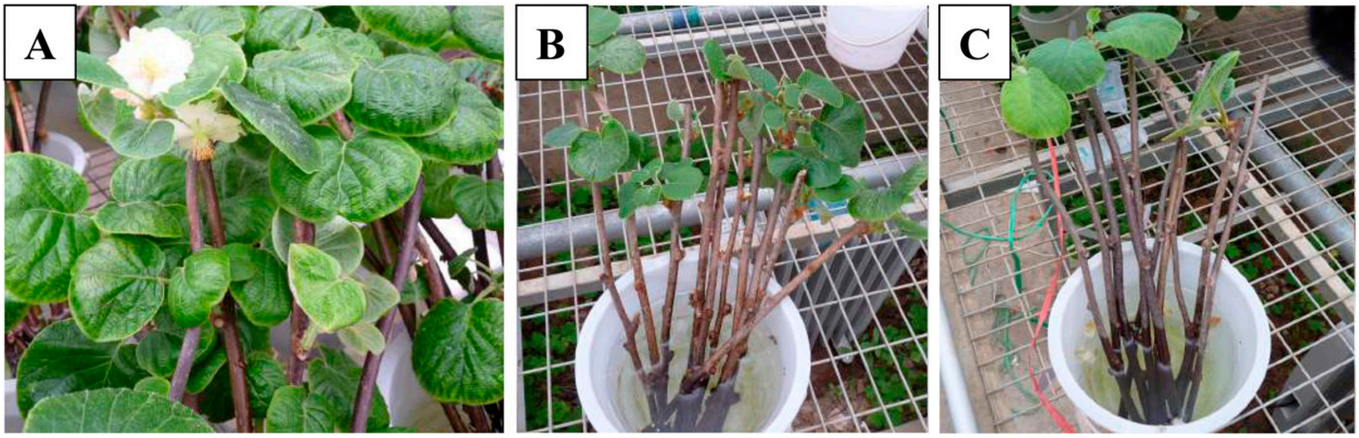

Fig. 1. The vegetative budbreak and floral emergence of three different kiwifruit cultivars with different ploidy levels at 24 chill portions, which corresponded to 271 chill units and 242 chilling hours. (**A**) Diploid cv. Donghong; (**B**) Tetraploid cv. H-15; (**C**) Hexaploid cv. Jinkui.

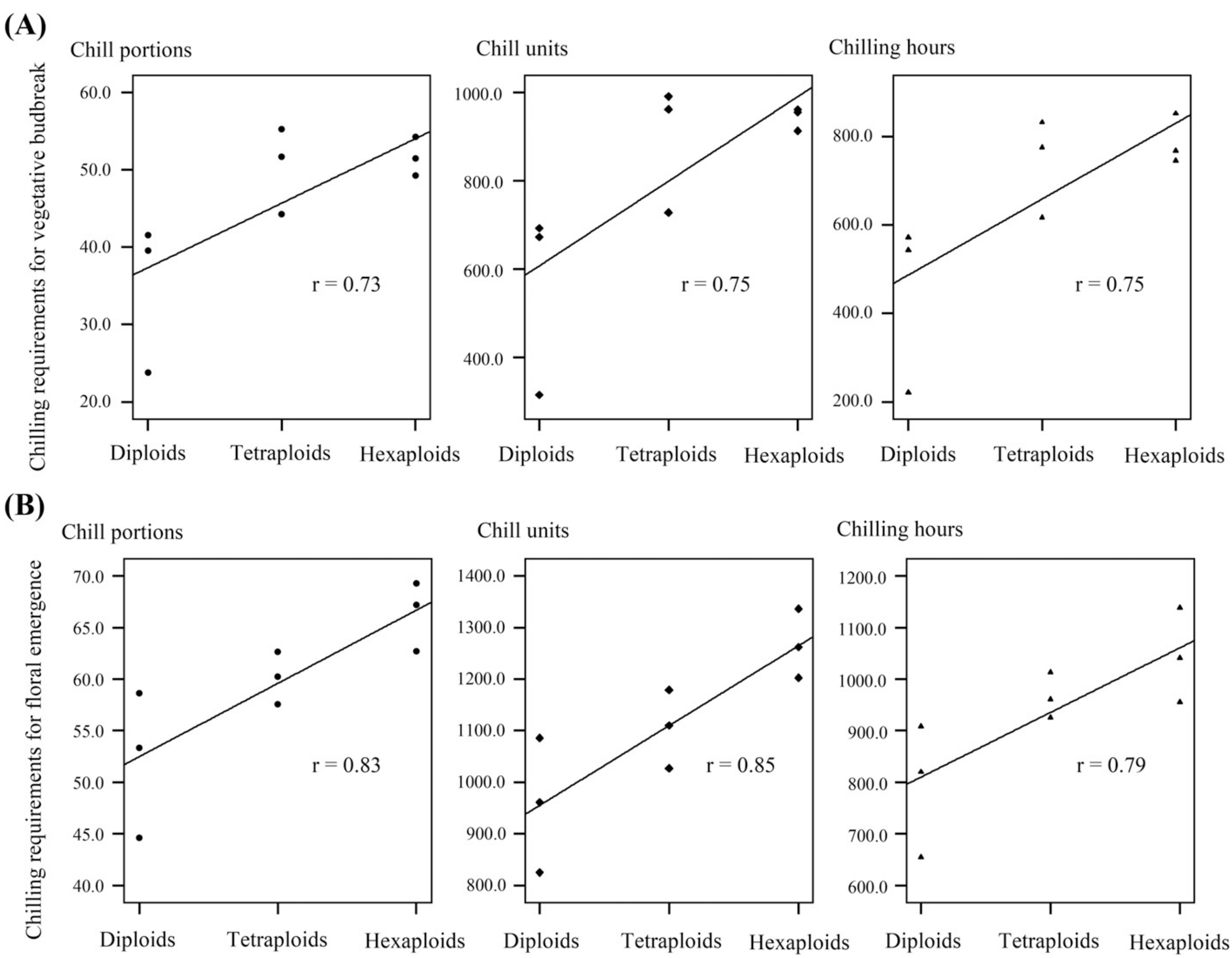

Fig. 2. The relationship of ploidy with chilling requirements for vegetative budbreak (**A**) and floral emergence (**B**). Significance of the regression coefficient was determined at $P < 0.05$. Each data point represents the mean value of a cultivar.

between ploidy levels and chilling requirements for vegetative budbreak and floral emergence across all models were 0.74 and 0.82, respectively (Fig. 2), indicating that both vegetative budbreak and floral emergence were positively related to the ploidy levels.

Discussion

In our field conditions, the correlation coefficient between portions (Dynamic model) and CU (Utah model) was higher than that between portions and CH (CH model) and between CU and CH (data not shown). Similarly, in estimating chilling requirements, the correlation between the Dynamic and Utah models ($r = 0.99$) is very high, which is similar to that reported in previous studies (Erez and Fishman, 1998; Ruiz et al., 2007). Hence, both the Dynamic and Utah models are suitable for estimating chilling requirements for kiwifruit cultivars in Wuhan. The correlations between the CH and Dynamic models ($r = 0.97$) were also quite high, as well as between the CH and Utah models ($r = 0.95$), indicating that the CH model could be a useful and reasonable method for growers.

The chilling requirement studies provide basic information for cultivation management and plant selection for specific sites (Dennis, 2003; Faust et al., 1991). In this study, the chilling requirements were estimated for kiwifruit cultivars with different ploidy levels. 'Donghong' showed the lowest chilling requirements and the highest break percentages for vegetative budbreak and floral emergence, revealing that it may be the most suitable cultivar for adaptation to warmer climates. Within the diploid level, considerable differences in chilling requirement were observed (Table 3), which indicates that selecting lower chilling or higher chilling materials is possible by interspecific or intraspecific hybrids. The chilling requirements of hexaploid cultivars were significantly higher than those of diploid cultivars, indicating hexaploids need higher chilling accumulation in winter than the diploids. Interploidy and intraspecific hybridization may be useful breeding strategies to improve the chilling requirement for specific growing zones. For example, Kataoka et al. (2014) selected selections below 300 CH by intraspecific hybridization of *A. chinensis* and *A. rufa*.

Conclusions

The kiwifruit cultivars examined in this study showed a wide range of chilling requirements for vegetative budbreak from 24 to 55 CP, 316 to 991 CU, and 222 to 853 CH. Floral emergence had higher chilling requirements than vegetative budbreak. A significant difference in chilling requirement was observed among the cultivars within the diploid level. The chilling requirements for vegetative budbreak and floral emergence in diploid cultivars were significantly lower than those in hexaploid cultivars with tetraploid cultivars intermediate. Significant correlations were determined between the ploidy levels and chilling requirements for vegetative budbreak and floral emergence. Our results provide baseline data for the breeding, selection, cultivation, and management of kiwifruit.

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