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Chilling requirement for seed germination and phenological observations on peach cultivars

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ABSTRACT

In subtropical climate areas, the models and methods proposed to evaluate the chilling requirement of temperate fruit crops often do not provide satisfactory results, thus calling for the development of alternative techniques. The aim of this study was to evaluate the correlations between some phenological traits and chilling requirement for seed germination of 18 peach cultivars and one nectarine cultivar. Two experiments were installed separately for the correlation studies. In experiment 1, the phenological traits were observed in the field, while in experiment 2, the chilling requirement for 50 and 100% seed germination of each cultivar was assessed. The number of days for beginning of bloom ($r = 0.70^{**}$, 0.61^{**}) and full bloom ($r = 0.72^{**}$, 0.76^{**}) were both significantly correlated with the number of chilling units for 50% and 100% germination of seeds. The number of days for beginning of budding and dormancy break were both significantly correlated with the number of chilling units for 50% and 100% germination ($r = 0.48^{*}$, 0.50^{*} , respectively). However, the same significant effect for these phenological traits was not found between chilling units and 50% germination of seeds, as well as between chilling units and harvest dates.

Key words: *Prunus* spp., peach, dormancy.

RESUMO

Necessidade de frio para estratificação das sementes e observações fenológicas em cultivares de pessegueiro

Em regiões de clima subtropical, os modelos e métodos propostos para avaliação da necessidade de frio das fruteiras de clima temperado não apresentam, muitas vezes, resultados satisfatórios. Isto demonstra a necessidade do desenvolvimento de alternativas para se estimar a necessidade de frio dessas plantas. Este trabalho teve como objetivo analisar as correlações entre os dados fenológicos e a necessidade de frio das sementes de 18 cultivares de pessegueiro e um cultivar de nectarineira. Para realização dos estudos de correlação, foram realizados dois experimentos, separadamente, sendo o primeiro por acompanhamento das características fenológicas em campo e, o segundo, com o estudo da necessidade de frio acumulado, para germinação de 50 e de 100% das sementes, de cada cultivar. Houve correlação significativa entre o número de dias necessários para o início do florescimento ($r = 0,70^{**}$, $0,55^{*}$) e plena floração ($r = 0,72^{**}$, $0,73^{**}$) e o número de unidades de frio acumulado, para germinação de 50 e de 100% das sementes estratificadas. O número de dias para o início da brotação e fim da dormência apresentou correlação significativa com o número de unidades de frio acumulado, para 50 e 100% de germinação ($r = 0,48^{*}$, $0,50^{*}$, respectivamente).

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Entretanto, o mesmo efeito significativo nestes dados fenológicos não foi observado para o número de unidades de frio acumulado, para 50% de germinação, como, também, entre o número de unidades de frio acumulado para estratificação das sementes e a época de colheita dos frutos.

Palavras-chave: *Prunus* spp., pêssego, dormência.

INTRODUCTION

A great deal of research and genetic improvement has been done on peach, leading to important advances in understanding the crop development (Barbosa *et al.*, 2010).

Selection in peach breeding programs is based on traits related to harvest potential and fruit quality to meet market needs, as well as adaptation to different climatic conditions (late frosts, chilling requirement, etc ...) (Moreno, 2005).

This fruit tree needs a certain amount of cold, varying with the cultivar, to break dormancy of seeds and buds resulting in seed germination and flowering. The cold triggers internal mechanisms, changing the nature and level of growth regulators involved in the control of the dormancy processes (Petri & Herter, 2004).

However, the climatic conditions of peach producing areas in Brazil vary considerably, particularly with respect to temperatures during the rest period of the trees. The areas suitable for peach cultivation in southeastern Brazil receive mild winters with large thermal oscillation with chill unit accumulation ranging from 50 to 300 (Citadin, 2001).

The conversion of average hourly temperature into chill units is not solely the sum of hours with temperatures below 7.2 °C, but a wider temperature range. The chill units accumulate daily until a particular cultivar reaches the end of dormancy.

The existing models and methods for evaluating the chilling requirements of plants in the field are generally used for agroclimatic zoning of the crop, but do not provide satisfactory results in many regions, which, in turn, can affect and impair the phenological evaluation. In these cases, the use of correlated traits may provide a better phenological characterization of individuals.

It is generally assumed that the basis for bud and seed dormancy is similar, ie, plants that have high winter chilling requirement produce seeds with the same requirement for stratification (Kester, 1969). However, the studies that led to this conclusion were performed with genotypes that need more chill hours, therefore the need to evaluate this process in cultivars with lower chilling requirement.

In case this is confirmed, the monitoring of seed germination in studies of endodormancy will facilitate selection in breeding programs, because it is a less cumbersome process than those involving whole plants.

This study aimed to analyze the correlations between the phenological traits days to full bloom, early bud development, break of dormancy and harvest, and the chilling requirements of seeds of 18 cultivars of peach and one cultivar of nectarine.

MATERIAL AND METHODS

The study was conducted in the Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa (MG), Brazil. We analyzed 18 peach cultivars: Aurora-1, Alô Doçura, Relíquia, Talismã, Rei da Conserva, Colibri, Campinas 1, Setembrino, Bolão, Cristal, Ouromel, Real, Biuti, Marli, Premier, UFV 186, Convênio and Topázio and the nectarine cultivar Josefina, in the 2003/2004 production year.

The studied cultivars were planted at the Experimental Station of the Universidade Federal de Viçosa, in the municipality of Araponga – MG, in 1991. The spacing between rows was 6.0 m and between plants was 4.0 m.

The experimental area is located within the coordinates 20° 40' south latitude and 42° 31' west longitude, altitude of 885 m and according to Köppen classification, the climate is of the Cwa type.

To perform the correlation studies, two experiments were conducted separately; Experiment 1 carried out the monitoring of phenological data in the field; and Experiment 2 studied the accumulated chilling requirements for induction of germination and germination of 50% and 100% of seeds of each cultivar.

Experiment 1

Eight mixed branches of the previous growth cycle of each cultivar, four per plant, with average length of 26 cm were individually identified for evaluation in the field under conditions of natural cold, before the dormant period (May 16). At this time, the total number of vegetative and flowering buds per branch was recorded. Weekly

evaluations were done during the study period through observation and count of terminal and lateral vegetative buds, with green buds and open flowers. The estimated break dormancy of each individual was based on the date on which 50% of the vegetative buds sprouted or were at the green bud stage (Raseira *et al.*, 1998). Full bloom was considered the time when 70% of flowers were open. Thus, data on the beginning of flowering and bud-burst, dormancy break, full bloom and harvest stage of each cultivar were recorded. Harvest stage was considered when fruit size reached maximum development and the green color of the skin background changed to yellowish-green or cream (Cantillano & Sachs, 1984). Throughout the experimental period, temperatures were recorded hourly using an R-704 RATONA thermo-surveyor. During the experiment, cultural practices (fertilization, fruit thinning, pest control and weeding), except for the application of chemicals to break dormancy and winter pruning were performed in the collection of peach trees in study.

Experiment 2

For the evaluation of chilling requirement, open pollinated peaches at the harvest stage were picked from the 18 peach cultivars and one nectarine cultivar in November and December 2003, at the Experimental Station Araponga (Table 1). After harvest, the fruits were taken to the Postharvest Laboratory at UFV, Viçosa-MG, washed and depulped. Seeds were removed by breaking the endocarp using a mini-vice, sterilized with a fungicide solution (Benlate 500 to 15 g L⁻¹) and placed in plastic bags (10 seeds/bag) containing filter paper soaked with the same fungicide solution used in the disinfection. The bags were sealed and taken to a cold room for stratification, at 5 °C (Selim *et al.*, 1998; Wagner Júnior *et al.*, 2006), relative humidity of 85 ± 2.5%, in the dark. The seeds were observed for the beginning of radicle protrusion every two days. At the end of this process, the number of accumulated chill units when the seeds reached 50% and 100% germination and the days required to initiate germination were recorded. The radicle protrusion was used as a reference to visibly mark the beginning of these processes. The experiment was arranged in a complete randomized design with four replications of ten seeds each. The number of days to the onset of germination and accumulated chill units required for germination of 50% and 100% of the seeds were subjected to analysis of variance and means were grouped by Scott-Knott ($\alpha = 0.05$) using the Genes software (Cruz, 2006). For conversion of temperature in chilling units, we used the model developed by Richardson *et al.* (1974), known as the Utah model. The model assigns one chilling unit for every full hour of a plant's exposure to temperatures between 2.5 and 9.1 °C.

Correlation analysis

Pearson correlation analysis was performed between the number of days (from 1st June) required to reach each phenological state of the plant in the field, and the number of accumulated chilling units for 50% and 100% germination.

RESULTS AND DISCUSSION

Experiment 1

Chilling requirement in the field:

The conversion of hourly temperatures during the experiment (Figure 1) into chilling units, using the Utah model (Richardson *et al.*, 1974), for all cultivars [Campinas 1 (-124.5 chilling units); Aurora-1, Biuti, Bolão, Colibri, Cristal, Real, Setembrino and Talismã (-134.5 chilling units); Ouromel (-161.5 chilling units); UFV 186 (-191 chilling units); Josefina' (-220.5 chilling units); Relíquia (-225.5 chilling units); Premier (-274 chilling units); Alô Doçura (-339 chilling units); Topazio and Convênio (-371 chilling units); Marli and Rei da Conserva (-485 chilling units)] showed that there was negative chill accumulation to break dormancy, which indicates that this model is not ideal for estimating the chilling requirement in the field in climatic conditions similar to the Southeast. This demonstrates the need to develop other methodologies or models to estimate the chilling requirement of both vegetative and flowering buds for the cultivars in these conditions.

Records on observations of the 18 peach cultivars and one nectarine cultivar phenological events showed that cv. Campinas 1 was the first to start flowering and reach full bloom and dormancy break; however, cv. Real began sprouting sooner than the others (Table 1).

Herter & Feliciano (1985) proposed that the early flowering can be used as an indicator of overall plant adaptation to climatic conditions in regions of low chill accumulation, and in this sense, besides cv. Campinas 1, the cultivars Aurora-1, Cristal, Ouromel, Josefina, Real, Relíquia, Setembrino, Talismã, Biuti, Colibri, UFV-186 and Topázio were more well adapted, starting their blooms in June.

In addition to the capacity of growing and flowering satisfactorily, peach trees grown in the southeast should produce early and good quality fruit. The early harvest is essential to improve the market price, since that enables offering the produce at a time of low supply, resulting in a better return to the grower.

Among the peach cultivars analyzed, Aurora-1, Real, Cristal, Setembrino, Marli, Premier, Colibri and Convênio reached to earlier harvest stage.

Albuquerque *et al.* (2000) evaluated 29 peach cultivars in the same production area during two years. Most of

these cultivars were also included in this study. They found that cultivars Tropical, Régis, Rubro-sol, Centenário, Premier, Centenária, Marli, Aurora-1, Aurora-2, Josefina, Relíquia, Setembrino, Alô Doçura, Maravilha, Okinawa and Talismã were early ripening. These results were similar to those obtained for cultivars Premier, Marli, Aurora-1, Setembrino, Josefina, Relíquia and Alô Doçura in our study.

Cultivar Marli, despite having the earliest fruit ripening, was the latest for flowering and bud-bursting, as well as to full bloom and dormancy break. This earliness resulted from the rapid fruit development cycle in this cultivar. The longest time for cv. Marli to enter into flowering and full bloom stages can be explained by the need for greater heat accumulation, since this cultivar is

less need for chilling accumulation compared with cv. Convênio (Raseira & Nakasu, 1998), which showed advanced flowering and bud differentiation (Table 1).

According to Citadin (1999), it is possible that peach cultivars exist with low winter chilling requirement to break dormancy and high heat requirement to induce anthesis.

Chill accumulation in temperate fruit species is necessary to overcome the bud dormancy and regain the ability to grow. However, for the resumption of growth, ie, bud differentiation and flowering, it should occur a certain amount of heat accumulation, varying with the cultivar (Citadin *et al.*, 2003).

In subtropical areas suitable for peach cultivation, the genetic improvement has pursued the development of

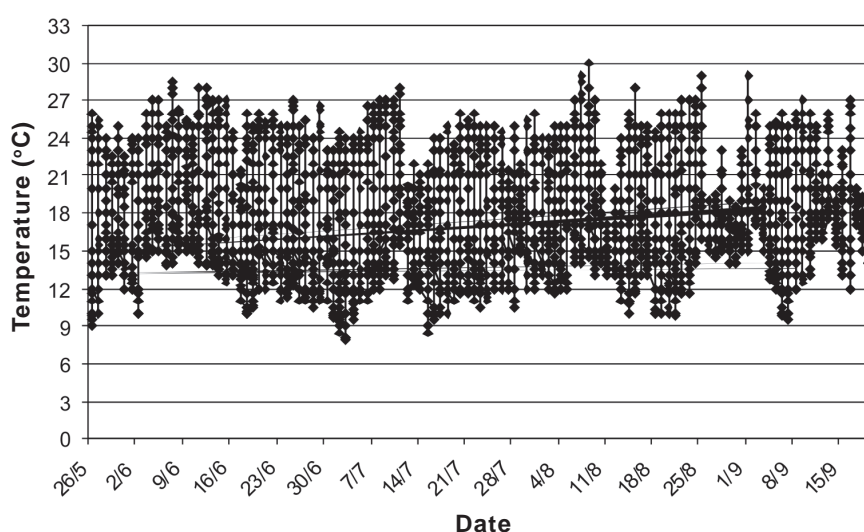


Figure 1. Hourly temperatures during the experimental period.

Table 1. Dates of early bloom, full bloom, bud-burst dormancy break and early harvest of 18 peach cultivars and one nectarine cultivar

Cultivar	Early bloom	Full bloom	Early budding	Dormancy break ¹	Harvest stage
Alô Doçura	04/07	11/7	29/8	5/9	14/11
Aurora-1	27/06	11/7	11/7	25/7	7/11
Biuti	27/06	18/7	4/7	25/7	21/11
Bolão	11/07	25/7	4/7	25/7	27/12
Campinas 1	14/06	20/6	4/7	11/7	14/11
Colibri	20/06	11/7	18/7	25/7	7/11
Convênio	27/06	15/8	5/9	12/9	7/11
Cristal	20/06	25/7	4/7	25/7	7/11
Josefina	27/06	15/7	1/8	15/8	14/11
Marli	15/08	19/9	12/9	19/9	7/11
Ouromel	27/06	1/8	25/7	1/8	14/11
Premier	11/07	15/8	22/8	29/8	7/11
Real	20/06	11/7	20/6	25/7	7/11
Rei da Conserva	11/07	15/8	5/9	19/9	5/12
Relíquia	27/06	18/7	18/7	22/8	14/11
Setembrino	20/06	18/7	11/7	25/7	7/11
Talismã	20/06	18/7	4/7	25/7	21/11
Topázio	11/07	22/8	5/9	12/9	14/11
UFV 186	27/06	1/8	25/7	8/8	5/12

¹50% of vegetative buds at the green bud stage.

cultivars with phenological traits similar to those of cv. Marli. The delay in flowering avoids losses caused by late frosts and allows that pollination and fertilization occur at times when the temperatures are more favorable. Our results allow the recommendation that cv. Marli should be used in future hybridizations within the peach breeding program of the Universidade Federal de Viçosa, as well as in others that aim for cultivars with late flowering and rapid fruit development cycle.

Experiment 2

Seed chilling requirement:

Analyses of variance showed that there were significant differences in the number of days required for the onset of germination and the accumulated chilling units needed to achieve 50% and 100% germination of the different cultivars. This shows that there is variability between the cultivars for these variables (Table 2). Variability is the necessary raw material used by the breeder in any breeding program.

Cultivars Campinas 1, Talismã and Ouromel required the shortest period (28 days) to start their germination, whereas cv. Bolão required the longest period (51 days) to begin the germination. Pérez-Gonzalez (1990, 1997) showed that the time to reach the onset of germination

(less than 35 days of stratification) has been used in the pre-selection and selection of genotypes with low winter chilling requirement in the subtropics. Considering this criterion, in this work, the cultivars Aurora-1, Alô Doçura, Campinas 1, Josefina, Ouromel, Real, Relíquia, Talismã, Biuti, Rei da Conserva, Colibri and Topázio, all starting germination before 35 days could be selected (Table 2). Our findings demonstrate the similar chilling requirements of these cultivars as already described by Antunes (1985). The cultivars Cristal, Setembrino, Premier and UFV 186 also had low winter chilling requirement and started germination between 36 and 40 days of stratification.

The cultivars that required less chilling accumulation to achieve 50% germination were Campinas 1 (796 CU), Rei da Conserva (870 CU), Josefina (898 CU), Ouromel (922 CU), Real (922 CU), Relíquia (952 CU), Aurora-1 (970 CU), Alô Doçura (976 CU), Talismã (978 CU), UFV 186 (1014 CU), Biuti (1055 CU), Colibri (1085 CU), Premier (1090 CU) and Cristal (1156 CU). On the other hand, cv. Marli had the highest chilling requirement to achieve 50% of germination, ie, 1786 CU. In addition, two intermediate groups were formed, Topázio and Bolão and Convênio and Setembrino, requiring 1540 CU and 1467 CU in the first group and 1318 CU and 1252 CU in the second group to achieve 50% germination, respectively.

Table 2. Number of days to start germination and accumulated chilling units (CU) for germination of 50% and 100% of seeds of 18 peach cultivars and one nectarine cultivar

Cultivar	Start of germination		CU**		Differences in CU (%)	
	Days	CU**	50% germination	100% germination	Start to 100% germination	50 to 100% germination
Aurora-1	34 d*	816	970 d	1156 c	340	186
Alô Doçura	31 e	744	976 d	1126 c	382	150
Campinas 1	28 f	672	796 d	922 c	250	126
Cristal	38 c	912	1156 d	1702 c	790	546
Josefina	31 e	744	898 d	1126 c	382	228
Ouromel	28 f	672	922 d	1204 c	532	282
Real	33 d	792	922 d	1246 c	454	324
Relíquia	31 e	744	952 d	1492 c	748	540
Setembrino	40 c	960	1252 c	1588 c	628	336
Talismã	28 f	672	978 d	1206 c	534	228
Marli	44 b	1056	1786 a	2246 a	1190	460
Premier	38 c	912	1090 d	1690 c	778	600
Biuti	34 d	816	1050 d	1440 c	624	390
Rei da Conserva	30 f	720	870 d	1092 c	—	—
Colibri	33 d	792	1086 d	1380 c	588	294
Bolão	51 a	1224	1467 b	1797 b	573	330
UFV 186	38 c	912	1014 d	1248 c	—	—
Topázio	31 e	744	1540 b	2313,75 a	—	—
Convênio	40 c	960	1318 c	2272 a	1312	954
CV (%)	5.13	13.63	22.86			

*Means followed by the same letters in the columns belong to the same group according to the test of Scott-Knott ($\alpha = 0.05$).

** Chilling unit calculated using the Utah Model (Richardson *et al.*, 1974),

The cultivars that needed the least accumulated chilling units to achieve 100% germination of seeds during stratification were Campinas 1, Rei da Conserva, Alô Doçura, Josefina, Aurora-1, Ouromel, Talismã, Real, UFV 186, Colibri, Biuti, Relíquia, Setembrino, Premier and Cristal. However, even with different times of stratification, all cultivars reached 100% germination.

The effect of stratification on the germination percentage observed in this study was also reported for other peach cultivars (Mehanna *et al.*, 1985; Wagner Junior *et al.*, 2006) and other species of the genus *Prunus* (Seeley & Damavandy, 1985). Diaz & Martin (1972) and Selim *et al.* (1998) found that during peach seed stratification at 5 °C, the content of growth promoters such as gibberellins and indole acetic acid increased in seeds and the content of inhibitors such as abscisic acid decreased.

Analysis of correlations

The cultivars UFV 186 and Rei da Conserva were not included in the analysis of the differences between the chilling requirement for initiating germination and for achieving 100% germination and between the chilling requirement to reach 50% and 100% germination, because the cultivars were selected locally by farmers and thus we had no information on their genealogy.

Significant correlation was found between the number of accumulated chilling units for germination of 50% and 100% of the seeds and the number of days from 1st. June to first bloom ($r = 0.70^{**}$; 0.55^{*}) and full bloom ($r = 0.72^{**}$; 0.73^{**}) (Table 3). This can be explained, in part, by the common characteristics of dormancy in seeds and buds that require low temperatures and the action of same growth regulators such as gibberellins, cytokinins etc., to overcome this process. Perez-Gonzales (1990) found, in Mexico, a significant correlation between flowering time and the number of days for 80% germination of seeds in local peach genotypes ($r = 0.71^{**}$) and genotypes introduced from other countries ($r = 0.87^{**}$) (United States, Italy, Spain and Brazil). Other studies also reported correlation between days to full bloom and seed chilling requirement for peach (Diaz & Martin, 1972; Chang & Werner, 1984; Guerriero & Scalabrelli, 1985) and almond (Kester, 1969; Kester *et al.*, 1977; Garcia-Worm *et al.*, 2004).

In studies carried out by Westwood & Bjornstad (1968) and Chang & Werner (1984), the extent of seed dormancy was associated with chilling requirement for buds of the female parent start sprouting, as well as buds from both parents, as highlighted by Hauagge (1988) and Hauagge *et al.* (1989). In this study, we also found for the female parent significant correlation between the accumulated chilling units to reach 100% germination and both the number of days for early bud formation from 1st. June (0.48^{*}) and dormancy break (0.50^{*}).

However, the phenological data on budding (early budding and dormancy break) showed no significant correlation with the number of chilling units for 50% germination ($r = 0.42^{ns}$ and $r = 0.40^{ns}$, respectively) (Table 3). In this case, the non-occurrence of correlation may be related to the greater variation in chilling requirement for 50% germination among the genotypes. This variation, if derived from the embryo genotype, may indicate the suitability of the technique to perform selection aiming at low chilling requirement.

There is also lack of significant correlation between accumulated chilling units required for 50% and 100% germination and the dates on which fruits were at the harvest stage (Table 3). This demonstrates that the fruit development cycle is independent of seed chilling requirements.

Albuquerque (1997), studying 29 peach cultivars, found no correlation between the flowering and harvest periods. The same fact was observed in this study, although not shown in Table 3 (early bloom x harvest stage ($r = 0.14^{ns}$) and full bloom x harvest stage ($r = 0.02^{ns}$)). The harvest stage depends on the time of flowering, but more accurately, on the period between flowering and ripening, which may vary between 60 and 200 days (Barbosa *et al.* 1990a; Barbosa *et al.* 1990b).

The differences between the chilling units required to start germination and to reach 100% germination and the differences between the chilling units required to achieve 50% and 100% germination show that the seeds of F1 cultivars (Campinas 1, Cristal, Real, Relíquia, Talismã, Marli, Premier, Biuti and Bolão) showed greater variability in both cases (660.11 CU and 393.78, CU respectively) when compared with seeds of F2 cultivars (Aurora-1, Alô Doçura, Josefina, Ouromel, Setembrino, Colibri and Convênio) (594.86 CU and 347.14, CU respectively) (Table 3).

Table 3. Correlations between accumulated chilling units required for 50% and 100% germination with five phenological traits of 18 peach cultivars and one nectarine cultivar

CU for germination	Early bloom	Full bloom	Early budding (Date)	Dormancy break ¹	Harvest stage
50%	0.70**	0.72**	0.42 ^{ns}	0.40 ^{ns}	0.04 ^{ns}
100%	0.55*	0.73**	0.48*	0.50*	- 0.12 ^{ns}

ns., **. *, non-significant, significant at 1 and 5% probability, respectively.

¹ 50% of vegetative buds at the green stage.

Seeds from F1 plants are already F2 generation and therefore will show segregation in relation to the hybrid loci of the F1 cultivars. Seeds of F2 cultivars will have hybrid constitution in fewer loci than F1 plants and, therefore, their seeds, which are F3, will have less variability than the first seeds. Ramalho *et al.* (2001) discussed that the F1 progeny is heterozygous for all homozygous loci in which both parents differ and, in the F2 generation, 50% of those loci will be heterozygous and 50% homozygous. This may explain the greater variability in the chilling requirements for germination of seeds from cultivars F1 and F2. Cultivar Topázio, although F2, showed the greatest difference between the chilling requirement to start germination and to achieve 100% germination. This may be due to its greater chilling requirement compared with the other cultivars, since it is more adapted to the climatic conditions of southern Brazil. Another possibility is that cv. Topázio has hybrid constitution in loci responsible for the chilling requirement.

CONCLUSIONS

Cultivar Campinas 1 was the first to bloom, to reach full bloom and dormancy break. Early fruit ripening was reached by cultivars Premier, Marli, Aurora-1, Setembrino, Josefina, Relíquia and Alô Doçura

Cultivars Campinas 1, Rei da Conserva, Alô Doçura, Josefina, Aurora-1, Ouromel, Talismã, Real, UFV 186, Colibri, Biuti, Relíquia, Setembrino, Premier and Cristal needed less accumulated chilling units for germination of all seeds.

Significant correlation was found between the chilling requirement for seed germination and phenological traits of blooming and between the chilling requirement for 100% germination and phenological data on budding.

There was no significant correlation between chilling requirements for germination and phenological data on peach harvest stage.

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