Investigation note

Effect of vernalization on strawberry stolon production

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Abstract

In strawberry cultivation, reproduction by stolons is the most used due to its low cost, the quantity and homogeneity of the daughter plants. Although it has the ability to produce stolons naturally, they can be exposed to vernalization periods to increase the yield and quality of planting material. This research aimed to determine the production of stolons and daughter plants of the varieties Albión, Festival and Oso Grande exposed to four vernalization temperatures under greenhouse conditions, in Heredia, Costa Rica, between September 2018 and February 2019. The following were quantified: the number and length of stolons; as well as the number of daughter plants per stolon, of 50 plants per variety, exposed to 0, 250, 500 and 750 cold hours at 6 °C. The three variables showed statistically significant differences with respect to the control. Plants of all varieties produced more stolons when vernalized. Festival obtained the highest number of plants per stolon with 250 cold hours (5) and Oso Grande with 500 cold hours had the longest stolon length, 143 cm. It was concluded that there is an effect of vernalization on the production of stolons and daughter plants depending on the variety.

Keywords: *Fragaria* × *ananassa* Duchesne ex Rozier, cold hours, vegetative propagation.

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In 2020, a world production of 8 861 381 t was estimated for strawberry crop, the largest producer was China with 37.5%, followed by the United States of America which produced 12%. Mexico, Spain, and Brazil together produced the same amount as the United States (FAOSTAT, 2022). In Costa Rica, strawberry is considered as a permanent crop that is in the hands of small producers, according to the latest Agricultural Census (INEC, 2015), there are 342 farms engaged in this crop, of which 151 have an area of less than one hectare and 121 farms are less than 5 ha. Costa Rica has been producing strawberries for more than 30 years, the most common varieties were Chandler and Oso Grande (Zumbado, 1994). Among the new varieties planted in Costa Rica are Albión, Cabrillo, Elyana, Festival, Portola, Real, Rubillen and San Andreas (MAG, 2021). According to Shaw and Larsson (2006), Albión, Elyana, Festival and San Andreas produce more flowers and fruits, which have greater firmness and flavor.

Initially, the daughter plants were obtained from the United States of America, then waste mother plants began to be brought from the same country. These remained for about two months at a temperature of 2 °C. This period of cold conservation made the plants stay alive for longer and receive the stimulus of cold necessary to start the production of stolons (Matamoros, 1996). Currently, in Costa Rica, with the development of new technologies, it is possible to produce stolons *in vitro* from mother plants. These seedlings could increase their quality, having the necessary cold to produce more stolons. This reduces production costs and time, in addition to providing plants with an acclimatization period similar to the one they will be exposed to when going out to the field (Jiménez and Alvarado, 2014), which could increase yield.

Costa Rica has comparative advantages in relation to other countries, being a tropical country, it can produce plants and fresh fruits throughout the year. This is unlike leading countries such as the United States of America, where the temperate climate makes the product scarce, mainly during the winter (FAO, 2002). The trial was carried out under greenhouse conditions in Santo Domingo de Heredia, Costa Rica at 1 360 masl, corresponding to a life zone called Premontane Wet Forest (wf-P). It began in September 2018 and was concluded at the end of February 2019. The production rows were placed from northeast to southwest in the same orientation as the multilayer semi-arch greenhouse, built with galvanized iron, covered with transparent polyethylene. The area of the experiment comprised 55.2 m^2 (Figure 1A).

All seedlings were obtained from *in vitro* culture to ensure quality, uniformity, and phytosanitary conditions. The planting was carried out in pots of 3 L, with coconut fiber substrate in a proportion of 70% coarse coconut and 30% fine coconut (2 mm and 0.5 mm, respectively). One plant was placed per pot, and they were installed in double row on cables at high of one meter. They were connected to the fertigation system (Figure 1B and C), with irrigation frequency of 5 times per day (from 9 am to 2 pm, every hour), with a duration of 3 min each.

The experiment consisted of 12 treatments derived from the factorial combination of three varieties (Albión, Festival and Oso Grande) and four vernalization durations (0, 250, 500 and 750 h) at 6 °C prior to establishment. The work was done with a total of 10 random repetitions per treatment, the experimental unit was one plant per pot.

Figure 1. Site of location of the trial. A) multilayer greenhouse completely covered with transparent plastic; B) beds of plants producing stolons; and C) plants newly sown in pots of 3 L.

In the three cultivars, the increase in cold hours caused an increase in the production of stolons per mother plant. The differences in the number of stolons were highly significant with respect to the controls (Table 1). There was a sustained upward behavior in the number of stolons in the varieties Albión and Oso Grande as the number of cold hours increased. These varieties also produced more stolons than the Festival variety. The total length of the stolon presented highly significant differences in relation to the control without vernalization for all varieties, in addition, there was a difference between varieties for 500 and 750 cold hours (Table 1).

Table 1. Average number and length (cm) of stolons and average number of daughter plants of strawberry (*Fragaria* × *ananassa*) Duchesne ex Rozier per stolon of the varieties Albión, Festival and Oso Grande exposed to 0, 250, 500 and 750 h of vernalization at 6 °C. Costa Rica. 2019.

Variety	Vernalization period (h) at 6 °C											
	0			250			500			750		
	Stolons		Dp	Stolons		Dp	Stolons		DP	Stolons		Dp
	Number	L (cm)		Number	L (cm)		Number	L (cm)		Number	L (cm)	
Albión	3^{Aa*}	62.53^{Aa*}	1.3^{Aa*}	4.8^{Ba}	115 77 Ba	2.9^{Ba}	8.8^{Cb}	133 07 Bab	3.5^{a}	9.9^{Cb}	128 30 Ba	2.9^{Ba}
Festival	1.7^{Aa}	87.3^{Aa}	2.6^{Ab}	6.1^{Cb}	12 370 Ba	5^{Cb}	4.7^{Ba}	117 50 Ba	3.5^{Ba}	6.6^{Ca}	110 17 Ba	3.7^{Ba}
Oso Grande	1.1^{Aa}	93.52^{Aa}	2^{Aab}	6.4^{Bb}	12 473 Ba	46^{Cb}	9.5^{Cb}	143 37 Cb	3.5^{Ba}	11.9^{Dc}	130 97 BCa	3.4^{Ba}

Dp= daughter plants; L= length. Averages compared by Anova, using a value of $p< 0.05$. *= different uppercase letters in the same row indicate significant differences between cold hours and lowercase letters highlighted in bold in the same column indicate significant differences between varieties.

With regard to the production of offspring per stolon, the plants without vernalization of the three varieties were the ones that produced the least. The Albión variety with 0 h of exposure to cold reported the lowest number of offspring production per stolon of all treatments (1.3); while Festival, at 250 h of cold, reached the highest value (5) (Table 1).

Studies on vernalization in strawberries have previously been carried out and have shown that exposure to cold improves some production characteristics, such as yield and number of fruits. In this regard, Rivera (1993) found that two weeks of vernalization (336 h between 0 and 5 °C) produced the highest yields per plant, while four weeks (672 h) decreased it by 50% and increased mortality. In this research, no significant differences were found between 500 and 750 h cold, so these plants probably do not present differences in yields.

Rodríguez-Bautista *et al*. (2012) found that plants of the Festival variety produce fewer stolons when exposed to colder climates due to altitudinal differences. These researchers obtained an average of 12 and 8.2 stolons/plant at an altitude of 1 700 and 2 228 m, respectively. These localities presented average annual temperatures of 17.7 °C and 16.2 °C, which indicates that the plants at 2 228 masl were exposed to more cold hours. This trend was observed when increasing from 250 to 500 h in this research, 6.1 and 4.7 stolons per plant, respectively; nevertheless, with 750 h the production of stolons (6.6) was statistically the same as with only 250 h. This indicates that the optimal vernalization value for this variety is 250 h, since it also produced stolons of the greatest length and with the largest number of daughter plants, which will probably result in higher yields.

With respect to the Albión variety, these authors found no differences in the number of stolons according to the planting altitude, 6.4 stolons/plant were produced in both localities, results different from those recorded in this study, where the number of stolons increased proportionally with the increase in cold hours. de Oliveira *et al*. (2017) studied the behavior without vernalization of ten strawberry varieties and found an inversely proportional relationship between the number of stolons and the number of daughter plants per stolon, the same behavior observed in this research. However, these authors found values between 3.9 and 8.4 stolons per mother plant, in the varieties studied, for example, Oso Grande produced 7.2 stolons per mother plant; while in this study, non-vernalized plants produced a maximum of three stolons, and it took at least 250 h cold to surpass the four stolons per plant.

According to Andrés *et al*. (2021), under natural conditions (without vernalization), short-day varieties that develop their crop cycle at 24 °C, such as Festival and Oso Grande with zero cooling hours, have the capacity to produce a high number of stolons per plant, due to the high level of expression of the FvGA20ox4 gene, essential for their development and which is regulated by the close relationship between photoperiod and temperature. Although the results did not show this behavior in the zero-hour treatment, an increase in the number of stolons was observed in the vernalized plants, which could indicate that the levels of expression of the gene vary according to the cooling hours of the plants.

Husaini and Wen Xu (2016) indicate that vernalization is an important factor in the vegetative and reproductive processes of strawberry plants and that it is necessary to break the dormancy of the buds and promote flowering, they also indicate that it is genotype dependent. In this sense, Strand

(2008) mentions that a mother plant has the capacity to produce up to 100 daughter plants in a crop cycle, which depends on the variety, locality, and agricultural practices. Lo anterior could explain the variation obtained between the cultivars studied.

According to da Costa *et al*. (2014), vernalization can predict the phenological cycle of day-neutral varieties such as Albión, they found that plants exposed to 4 °C for 24 days (576 h) flowered 20 days earlier than non-vernalized plants, which presupposes that they produced fewer stolons; since cooling before planting is a strategy to stimulate floral differentiation over the vegetative one (Diel *et al*., 2017).

The above suggests that it is more useful, in terms of vegetative propagation, for the mother plant to have few productive stolons than large numbers of stolons with few daughter plants. This, in addition to increasing the production of propagules, in turn provides a more aerated and cleaner environment, somewhat reducing the presence of pests and diseases. In addition, it makes it easier for the producer to spray and harvest (IICA, 2017).

Conclusions

According to the findings of this research, vernalization positively impacts the production of strawberry stolons and daughter plants of the Albión, Festival and Oso Grande varieties.

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