DOI: 10.5433/1679-0359.2018v39n6p2325

Influence of seed vernalization on production, growth and development of lisianthus

Influência da vernalização de semente na produção, crescimento e desenvolvimento de plantas de lisianthus

Maria Yumbla-Orbes[1*]; José Geraldo Barbosa[2]; Wagner Campos Otoni[3]; Marcel Santos Montezano[4]; José Antônio Saraiva Grossi[2]; Paulo Roberto Cecon[5]; Eduardo Euclydes de Lima e Borges[6]; Joice Crescencio Heidemann[4]

Abstract

Flowering induction and control is a limiting factor when commercially producing cut flowers of lisianthus and seed exposure to low temperatures, a physiological event called vernalization, induces the differentiation of vegetative buds to reproductive buds, contributing to a flowering that is uniform and has quality. The objective of this study was to evaluate the influence of seed vernalization in three cultivars of lisianthus (Excalibur, Echo and Mariachi) for 12, 24, 36 and 48 days at temperatures of 5, 10 and 15°C, in the production and quality of buds, making this technology feasible to large-scale production. During cultivation it was observed that the lower the temperature and higher the vernalization period, the lower the cycle and the greater the number of plants induced to flowering for all three cultivars, and those are important features in the context of flower production in a commercial scale. The seeds subjected to vernalization originated plants that produce flower stems within the standards required by the market, showing that vernalization was efficient to induce flowering without affecting the quality of the buds. To produce lisianthus as a cut flower of quality, it is recommended seed vernalization of Mariachi and Echo cultivars for 24 days at 5°C and Excalibur for 36 days at 5°C.
Key words: *Eustoma grandiflorum.* Seed vernalization and flowers.

Resumo

A indução e controle do florescimento é fator limitante na produção comercial de flores de corte em lisianthus e ocorre pela exposição das sementes a baixas temperaturas, evento fisiológico denominado vernalização, o qual induz a diferenciação das gemas vegetativas em reprodutivas, contribuindo para uma floração uniforme e de qualidade. Assim, o objetivo deste estudo foi avaliar a influência da vernalização de sementes de três variedades de lisianthus (Excalibur, Echo e Mariachi), por 12, 24, 36 e 48 dias, a temperaturas 5, 10 e 15 ° C, na produção e qualidade das hastes florais e disponibilizar esta

[1] Profª Pesquisadora, Facultad de Ciencias Agrícolas, Ingeniería Agronómica, Universidad Central del Ecuador, Quito, Ecuador. E-mail: maria.yumbla@hotmail.com; myumbla@uce.edu.ec

[2] Profs. Pessquisadores, Departamento de Fitotecnia, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brasil. E-mail: jgeraldo@ufv.br; jgrossi@ufv.br

[3] Prof. Pessquisador, Departamento de Biologia Vegetal, Laboratório de Cultura de Tecidos/BIOAGRO, Centro de Ciências Biológicas e da Saúde, UFV, Viçosa, MG, Brasil. E-mail: wotoni@ufv.br

[4] Pesquisadores, Departamento de Fitotecnia, UFV, Viçosa, MG, Brasil. E-mail: marcelsanmon@gmail.com; sunjoyflower@gmail.com

[5] Prof. Pessquisador, Departamento de Informática, UFV, Viçosa, MG, Brasil. E-mail: cecon@dpi.ufv.br

[6] Prof. Pesquisador, Departamento de Engenharia Forestal, UFV, Viçosa, MG, Brasil. E-mail: elborges@ufv.br

[*] Author for correspondence

Received: June 19, 2017 - Approved: Aug. 15, 2018

tecnologia ao sistema produtivo. Ao longo do cultivo observou-se que quanto menor a temperatura e maior o período de vernalização, menor foi o ciclo e maior foi o número de plantas induzidas ao florescimento, para as três cultivares, características importantes no contexto da produção de flores em escala comercial. As sementes submetidas à vernalização originaram plantas que produziram hastes florais dentro dos padrões exigidos pelo mercado, mostrando que a vernalização foi eficiente no sentido de indução das plantas ao florescimento sem afetar a qualidade das hastes florais. Para o cultivo de plantas de lisianthus como flor de corte de qualidade recomenda-se vernalizar as sementes das cultivares Mariachi e Echo por 24 dias a 5 °C e da Excalibur por 36 dias a 5 °C.
Palavras-chave: *Eustoma grandiflorum.* Vernalização de sementes e flores.

Introduction

Lisianthus (*Eustoma grandiflorum*) originated in the southern United States and northern Mexico and was introduced in Brazil in the late 80's becoming very popular as cut flower and potted plant. It is an herbaceous species with upright stems that depending on the cultivation technique can have an annual or a biennial cycle, being grown as an annual plant, requiring mild temperatures to have a good development (LORENZI; SOUZA, 2008). Thus, in winter, it has slow growth with rosette formation; branch stretching occurs in spring and flowering in summer (DOLE; WILKINS, 2005).

Lisianthus shows wide genetic variability with important features such as growth rate, leaf shape, color of flowers, inflorescence morphology, ethylene sensitivity and flowering cycle (ALVES et al., 2015). It also shows sensitivity to photoperiod and temperatures that are relevant environmental factors in flowering induction. Thus, exposure cultivars of lisianthus to high temperatures during and after seed germination can lead to the induction of plant with a rosette formation and flowering inhibition.

Rosette formation is a physiological disorder that inhibits elongation of internodes, with leaves growing radially and very close together. This results in a compact unit or in rosetted leaves, characterized by reduced internodal growth, not occurring emission of inflorescences and affects commercial production of flowers (TAIZ; ZEIGER, 2013).

In order to avoid rosette formation, it is necessary cultivation in a temperature average of 23/18°C day/night, respectively, until emergence of second or third leaf pairs, since after this stage the plant susceptibility decreases (ROH et al., 1989; HARBAUGH; ROH, 1992). Another technique to monitor this physiological disorder is exposure of seeds or seedlings to certain cold periods, a phenomenon called vernalization, that is a physiological event by which flowering is promoted by using a cold treatment in hydrated seeds or growing plants. Without this cold treatment, plants requiring vernalization delay flowering or remain in a vegetative stage (TAIZ; ZEIGER, 2013). At the same way, the bulbification of onion (*Allium cepa* L.) only begins when the combination of photoperiod and temperature whitch are determinants for bulbification of each cultivar is reached. It can be grown in tropical regions with the use of vernalization of the bulbs (SANTOS et al., 2012).

Studies on seed vernalization (KAGEYAMA et al., 1990; PERGOLA et al., 1992; TANIGAWA et al., 2002) as well as on plant vernalization (HARBAUGH; ROH, 1992; HARBAUGH, 1995) have been proposed for different cultivars of lisianthus. Among the advantages found on vernalization of lisianthus seed is an increased seed germination rate, higher percentage of plants induced to flowering, more uniform seedlings and with high quality, which will reflect in the quality and productivity of plants and greater appreciation by the market, resulting in higher economic yield to the producer.

In commercial production of lisianthus, seed and seedling vernalization is used in order to avoid rosette formation (SAKATA, 2012) allowing stem elongation and plant flowering after seedling transplantation. Due to high genetic diversity in lisianthus it is not known an effective temperature range for seed vernalization, suggesting it to be between 1 ° C and 10 ° C. Vernalization requires several weeks of exposure to low temperatures, and it depends on the species and cultivar (KIM et al., 2009), indicating the need for research in order to establish the most appropriate handling of lisianthus varieties in order to obtain higher flower yields and quality. This work aimed to evaluate the influence of hydrated seeds to vernalization temperatures for different periods in growth and development three cultivars of lisianthus, as well as to provide a practical technology for seedling production and lisianthus cultivation as cut flower.

Material and Methods

Experiments were conducted in a randomized block design in a factorial arrangement of 3 x 4 + 1 additional treatment, three cultivars were studied: Excalibur White, Echo Lavender and Mariachi, F1 (Sakata Seed®). Cultivars were chosen by their high market acceptance, as well as the fact that they have different levels of sensitivity to rosette formation. According to sensitivity to high temperatures, lisianthus cultivars were classified as sensitive, not very sensitive and tolerant, growing in form of rosettes, semi-rosette or normal, respectively (FUKUDA et al., 1994; HARBAUGH; SCOTT, 1999). Mariachi and Echo cultivars are early and sensitive to rosetting cycle; Excalibur cultivars, with medium cycle, has low sensitivity to rosetting (SAKATA, 2010).

Seeds were exposed to different vernalization temperatures (5, 10 and 15°C) by four periods (12, 24, 36 and 48 days) using three replicates and three plants each. Further treatment consisted of unexposed seeds to vernalization temperatures for each variety.

Seeds were sown in plastic trays with 288 cells (18 ml cell: 2 x 2 x 4.5 cm) placing one seed in each cell, which was filled with Bioplant® commercial substrate. After sowing, they were wetted and covered with plastic bags to maintain high humidity and covered with aluminum foil to avoid light exposure. Thereupon, the trays were placed in BODs in accordance with the established vernalization temperature and period. After exposure to cold, depending on the treatment, seeds were germinated in BODs at 25°C, covered with plastic. Then they were transferred to a seed bed, covered with plastic and shading with restriction of 50% light to seedling growth. In this step, irrigation was with micro-sprinklers via automated system applying irrigation for 2 seconds at every 15-30 minutes according to their needs. Commercial fertilizer (Peters Professional®) containing 20% N; 20% P_2O_5; 20% K_2O, plus micronutrients was applied after emergence of cotyledons at 0.125 g L^{-1} rate twice a week.

Seedlings were transplanted to 10 L pots when they presented four or five pair of leaves. Pots were filled with 9 L of a mixture containing Bioplant® commercial substrate, sand and carbonized rice husk, at a 6:2:3 ratios, respectively. At this point 1 g L^{-1} fertilizer (10-10-10) was added being transplanted 3 plants per pot. Pesticide application was performed when needed. Plant fertirrigation was weekly with *B&G Flores Violeta* fertilizer containing 6.2% N; 4.1% P_2O_5; 8.2% K_2O; 2.5% Ca; 0.58% Mg; 1.8% S and 3.1% Cl, plus micronutrients.

The experiment was conducted from December/2013 to June/2014, during which the daily meteorological data of maximum and minimum temperatures were recorded in a Datalog installed in the center of the greenhouse. The photoperiod was calculated by the Cooper equation (COOPER, 1969).

Evaluations were: plants induced to flowering (%); flowering cycle in days (expressed by the period from seedling planting until harvesting);

stem length, in cm, measured 2 cm above the surface of the substrate to the stem apex; rod base diameter, in mm, measured 2 cm above the substrate surface; fresh and total dry mass per rod in g and number of flowers per stem. To determine dry mass, parts were separately placed in paper bags, and then placed in an oven at 60°C for 96 h and after this period they were weighed on a precision scale.

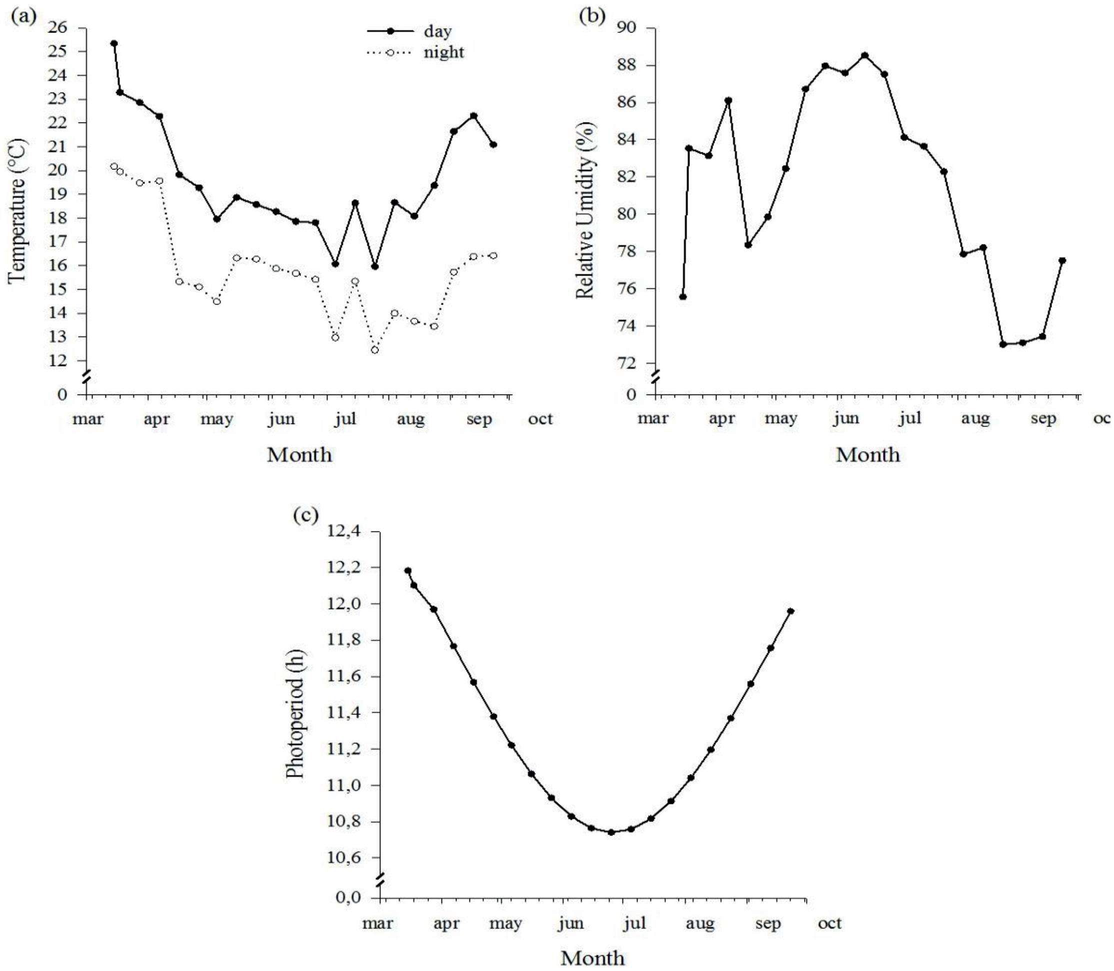
All variables were subjected to analysis of variance, and when significant, the averages were compared using the Tukey test at 5% significance. Comparison with the additional control treatment was carried out by Dunnett test at 5%. Statistical analyzes were performed using the R software version 3.0.3 (R CORE TEAM, 2014) with ExpDes package version 1.1.2 (FERREIRA et al., 2013) and Easyanova package (ARNHOLD, 2014). Graphs were plotted in Sigma Plot® software (version 11.0).

Results and Discussion

Characterization of the crop environment

In the development phase, the average diurnal temperature was 19.8°C and 15.9°C at night (Figure 1a). In turn, the maximum photoperiod in this phase was 12.22 h/day in March, and the minimum was 10.73 h/day in June (Figure 1b).

Figure. 1. Variation of day and night temperatures (a), relative humidity (b) and photoperiod (c) at the *E. grandiflorum* reproductive stage.



Flowering induction

Plants from vernalized seeds of cv. Mariachi presented means values for percentage of plants induced to flowering between 89 and 100%, which were higher than the control (66.7%), with the exception of plants from seeds that were vernalized for 12 days at 10 to 15°C and 48 days at 15°C, with average values of 77.8; 77.8 and 66.7%, respectively (Table 1) were similar to those observed in plants from seeds not subjected to cold.

Table 1. Flowering plants (FP) (%)**,** based on vernalization time and temperature for *E. grandiflorum* cv. Mariachi and Echo seeds.

Tratamentos	Mariachi	Echo
Control	66.7	88.9
12d 5°C	88.9*	77.8^{ns}
24d 5°C	100*	100^{ns}
36d 5°C	100*	88.9^{ns}
48d 5°C	100*	100^{ns}
12d 10°C	77.8^{ns}	77.8^{ns}
24d 10°C	100*	55.6^{ns}
36d 10°C	88.9*	88.9^{ns}
48d 10°C	100*	100^{ns}
12d 15°C	77.8^{ns}	66.7^{ns}
24d 15°C	100*	33.3*
36d 15°C	88.9*	22.2*
48d 15°C	66.7^{ns}	55.5^{ns}

Means followed by the asterisk in column differ from the control by Dunnett test at *P<0.05 probability.

Higher percentage of induced plants, 97.2%, was observed in plants of Mariachi cultivars whose seeds were vernalized at 5°C, similarly to those vernalized at 10°C (91.7%). In turn, seeds that remained for 24 days under vernalization, regardless the temperature, induced flowering in 100% of plants, not differing from plants grown from seeds that remained for 36 or 48 days under vernalization (Table 2), showing that this cold period was sufficient for the induction of seed to flowering.

Table 2. Flowering plants (FP) (%), based on vernalization time and temperature for *E. grandiflorum* cv. Mariachi.

Temperature		**Period**	
5 °C	97.2 a	12 d	81.5 b
10 °C	91.7 ab	24 d	100 a
15 °C	83.3 b	36 d	92.6 ab
		48 d	88.9 ab

Means followed by the same letters do not differ by Tukey test at *P<0.05 (*) probability.

Cold treatment for 24 days at 5°C; 48 days at 5°C or 48 days at 10°C applied to seeds of cv. Echo provided 100% plants induced to flowering and showed no difference from plants originated from not vernalized seeds, although there was flowering in only 88.9% of them (Table 1), which is undesirable at a market level.

Seeds from cv. Echo, vernalized at 5°C for 12, 24, 36 and 48 days instigated flowering of 77.8, 100, 88.9 and 100% plants, respectively, while in plants from seeds vernalized at 10°C, values were 77,8; 55,6; 88,9 and 100%. Plants from seeds submited to temperature of 15°C had low efficiency regarding flowering, becoming the limiting value in the commercial production of lisianthus, thus seeds of this cultivar need lower temperatures in order to obtain greater flowering efficiency (Table 3). In this context, Xi-heng et al. (2011), observed that vernalization of hydrated lisianthus seeds at 7 - 10°C increased germination rate and the percentage of plants induced to flowering.

Table 3. Flowering plants (FP) (%), based on vernalization time and temperature for *E. grandiflorum* cv. Echo.

	5 °C	10 °C	15 °C
12 d	77.8 Aa	7.,8 Aab	66.7 Aa
24 d	100 Aa	55.6 Bb	33.3 Bab
36 d	88.9 Aa	88.9 Aab	22.2 Bb
48 d	100 Aa	100 Aa	55.6 Bab

Means followed by the same lowercase letters vertically do not differ by Tukey test at *P<0.05 (*) probability.
Means of control followed by the asterisk differ for the treatments by Tukey test at **P<0.01 or *P<0.05 probability.

No differences were seen in the number of plants that flowered regarding temperature and vernalization period, but seeds from cv. Excalibur submitted to 5 and 10°C had 100% plants flowering, while non vernalized seeds presented 89% plants flowering.

Control of flowering is a limiting factor when commercially producing cut flowers of lisianthus and it occurs by seed exposure to low temperatures, since under inappropriate temperatures the plants present rosette formation, whose effects are inhibition of flowering, increased crop cycle and reduced stem quality (AMASINO, 2005; KOBORI, 2012). Throughout cultivation it was observed differential behavior in the response of cultivars to vernalization, but it was found that the lower the temperature and the longer the seed vernalization time, the greater the percentage of plants induced to flowering, and this was observed for all three cultivars. Thus, the period of exposure to cold and vernalization temperature are important aspects that need to be considered when applying low temperatures, as shown by Oka et al. (2001) with lisianthus, that observed a positive correlation between the vernalization time and rate of seedlings induced to flowering, the same was observed in this study.

Flowering cycle

The cultivation cycle of plants grown from non-vernalized seeds of cv. Mariachi was 190.7 days, and this value was higher than those observed in plants grown from vernalized seeds, whose average cycles varied from 147 to 167 days (Table 4). There were significant differences between plants from non-vernalized and vernalized seeds of cv. Excalibur, as to this feature, there was no decrease in crop cycle with seed vernalization in plants from non-vernalized seeds, when applying the following treatments: 36 and 48 days at 5°C (161.8 and 157.6 days) 12 days - 10 and 15°C (160.3 and 163.6 days) and 24 days at 15°C (164.1 days), as shown in Table 4, expressing the importance of exposing seeds to cold in early production of lisianthus flowers.

Table 4. Crop time (CT) (days), based on vernalization time and temperature for *E. grandiflorum* cv. Mariachi and Excalibur seeds.

Treatments	Mariachi	Excalibur
Control	190.7	188.7
12d 5°C	154.3*	166.9^{ns}
24d 5°C	151*	165.7^{ns}
36d 5°C	147.1*	161.8*
48d 5°C	149.6*	157.6*
12d 10°C	167.5*	160.3*
24d 10°C	153*	173.1^{ns}
36d 10°C	152.8*	160.1*
48d 10°C	150.2*	160.9*
12d 15°C	163.6*	163.6*
24d 15°C	150.4*	164.1*
36d 15°C	158.4*	165.3^{ns}
48d 15°C	151.7*	172.7^{ns}

Means followed by the asterisk in column differ for the control by the Dunnett test at *P<0.05 probability.

Plants from seeds of cv. Mariachi vernalized for 12 days showed higher crop cycle average, 161.8 days, and plants from seeds subjected to periods of 24, 36 and 48 days of cold had crop cycles of 151.48, 152.8 and 150.5 days, respectively (Table 5). The cultivation cycle of plants from seeds of cv. Echo, vernalized at 5°C (171.3 days) was shorter when compared with plants derived from seeds vernalized at 10 and 15°C (181 and 188.6 days, respectively), having no difference when compared to seeds vernalized at 10°C. Regardless the temperature, the shorter cycle occurred in plants grown from seeds that were vernalized for a period of 48 days (172.8 days), a value that is similar to that of seeds vernalized for 24 or 36 days (Table 4) and lower than plants from seeds vernalized for 12 days, showing the need of exposing seeds to at least 24 days of cold.

Plants were classified in three categories according to their growing cycle: <160 days; 160-180 days and >190 days. It was found that vernalization of cv. Mariachi seeds at 5 °C, 10°C or 15°C led to a reduction in the plant cycle, yielding over 75% rods collected with cycle equal to or less than 160 days, except in seeds vernalized for 12 days at 10°C or 12 or 36 days at 15°C, while the growing cycle of non-vernalized plants exceeded 190 days in over 75% plants.

For cv. Echo was observed that seeds vernalized at 5°C for 24, 36 or 48 days enabled 50, 42.8 or 37.5% plants collected with cycle <160 days; this was also observed in plants grown from seeds vernalized at 10°C for 48 days. However, in plants from non-vernalized seeds, only 14.1% stems were collected with cycle <160 days. Also, 75% of plants from cv. Excalibur seeds vernalized at 5°C for 48 days were collected with cycle below or equal to 160 days. Vernalization at 10 or 15°C increased the percentage of plants collected before 180 days, whereas non vernalized seeds yielded a higher percentage (62.5%) of plants with crop cycle >190 days.

Table 5. Total fresh weight/stem (TFW) (g), total dry weight/stem (TDW) (g) and number of open flowers/stem (NOF) based on vernalization time and temperature for *E. grandiflorum* cv. Mariachi seeds and total fresh weight/stem (TFW) for Excalibur seeds.

Treatments	TFW	TDW	NOF	TFW/excalibur
Control	91.6	14.2	16.3	88.6
12d 5°C	74.4^{ns}	13.5^{ns}	13.6^{ns}	83.7^{ns}
24d 5°C	58.5^{ns}	9.7^{ns}	11.4^{ns}	82.2^{ns}
36d 5°C	62.4^{ns}	9.9^{ns}	9.31^{ns}	93.4^{ns}
48d 5°C	58.9*	9.6*	10.9^{ns}	72.8^{ns}
12d 10°C	69.9^{ns}	11.9^{ns}	13.5^{ns}	53*
24d 10°C	66.9^{ns}	10.4^{ns}	12.6^{ns}	89.3^{ns}
36d 10°C	60.5^{ns}	9.7^{ns}	12.9^{ns}	85.1^{ns}
48d 10°C	55.9*	8.5*	8.9*	83.4^{ns}
12d 15°C	73.1^{ns}	11.9^{ns}	14.8^{ns}	88.6^{ns}
24d 15°C	57.1*	8.9*	10.6^{ns}	89.9^{ns}
36d 15°C	67.5^{ns}	10.9^{ns}	11.2^{ns}	96.2^{ns}
48d 15°C	61.9^{ns}	9.3*	9.2^{ns}	93.3^{ns}

Means followed by the asterisk in column differ for the control by the Dunnett test at *$P<0.05$ probability.

It was evident that vernalization led to standardization and reduction in the cultivation cycle and seed vernalization at 15°C was less effective when compared to the temperatures of 5°C and 10°C for the three cultivars. The earliness of flowering was most striking in cvs. Mariachi and Excalibur, whose average cycles were 151 and 164 days when seeds were vernalized at 5°C for 48 days showing a reduction of 40 and 27 days when compared to cycles of plants from non vernalized seeds which were 191 and 189 days. In the same way, these results show that temperature and duration of cold exposure, required to reach the state of vernalization, vary between species and cultivars (KIM et al., 2009).

In commercial cultivation of cut flowers it is very important for producers to reduce the crop cycle and standardize harvest. Studies have shown that vernalization may be a strategy to achieve these goals in several species, as shown by experiments on vernalization performed in *Brunonia australis* (CAVE; JOHNSTON, 2010), *Rhodanthe Floribunda* (HA; JOHNSTON, 2013) and *Lilium longiflorum* (HAMO et al., 2015) and as evidenced in this work for the three cultivars of lisianthus. Thus, Tanigawa et al. (2002) vernalized imbibed seeds of lisianthus of cvs. Asukanoasa and Asukanosakura at 11°C for 0, 1, 3 or 5 weeks, and found an increase in the germination rate and flowering induction with increasing vernalization time, as well as reduction in flowering cycle in comparison with plants obtained from not vernalized seeds, showing that vernalization becomes more effective with prolonged exposure to low temperatures, as occurs in nature (AMASINO, 2005) and was confirmed in this study.

Quality of stems

Fresh mass production of floral stems from non-vernalized seeds of cv. Mariachi was 91.6 g, differing from those observed on the stems of plants from seeds that were vernalized for 24 or 48 days at 5 °C, for 48 days at 10 °C and 24 days at 15 °C. Dry mass average value for control was 14.2 g, this value is higher than what was observed in stems of vernalized plants, except from the seeds vernalized for 12, 24 and 36 days at 5 or 10 °C and 12 and 36 days at 15 °C. The number of open flowers per stem in plants from non-vernalized seeds, 16.3, was similar to that observed in plants from vernalized seeds, except for the number of flowers in plants from seeds vernalized for 48 days at 10 ° C, 8.9 (Table 5).

Plants from cv. Excalibur seeds vernalized for 36 hours at 5 and 15 °C and for 48 days at 15 °C resulted in 93.4, 96.2 and 93.3g of fresh weight of stem, values similar to what was observed in the control treatment, 88.6 g (Table 5).

Dry mass had significant differences among the values obtained during the periods of vernalization, regardless of temperature, and seeds from cv. Mariachi that remained 12 days in cold had higher average for total dry matter (12.44 g), a similar value was observed for stems of plants coming from seeds vernalized for 36 days. Exposure of seeds to vernalization for 12, 24 and 36 days resulted in a production of 13.9, 11.5 and 11.2 flowers per stem, while the vernalization for 48 days reduced the number of flowers to 9.7 (Table 6a). In the cv. Echo was observed average stem length values of 52.3, 49.7 and 45cm in plants from seeds vernalized at 5, 10 and 15 °C respectively, and seeds exposed to temperatures of 5 and 10 °C had similar values (Table 6b).

Table 6. Growth and development characteristics based on vernalization time and temperature for *E. grandiflorum* cvs. Mariachi and Echo.

(a) cv. Mariachi

Period	Number of open flowers/stem	Total dry weight/stem (g)
12 d	13.9 a	12.4 a
24 d	11.5 ab	9.7 b
36 d	11.2 ab	10.2 ab
48 d	9.7 b	9.1 b

(b) cv. Echo

Temperature	Stem length (cm)
5 °C	52.3 a
10 °C	49.7 ab
15 °C	45.4 b

Means followed by the same letters do not differ by the Tukey test at *P<0.05 (*) probability.

In addition to the induction of flowering and cycle reduction, vernalization may affect the quantity and quality of flowers/inflorescence (HA, 2014), mainly expressed by the length, fresh and dry mass production and number of flower per stem. In this context, non-vernalized seeds of cultivar Mariachi generated plants with more open flowers, stem diameter, leaf area and fresh mass compared to plants from vernalized seeds, regardless the temperature. The same happened with other cultivars, showing that vernalization of lisianthus seeds did not increase the stem quality. This behavior is due to a longer cultivation cycle of plants from non-vernalized seeds, which were exposed to low temperatures, naturally occurring differentiation from vegetative buds to reproductive buds.

According to the pattern for lisianthus proposed by the *Cooperativa Veiling Holambra*, stems must have a minimum length of 40 cm, a minimum diameter of 4 mm, and the cutting packet of lisianthus should have at least 8 stems, reaching a weight of 500 g. So even with the differences in the phytotechnical characteristics assessed regarding cold treatments and genetic variability, seeds subjected to vernalization originated plants that produce flower stems within the patterns required by the market, showing that vernalization was efficient in flowering induction and reducing crop cycle without impairing the stem quality.

Conclusions

Seed vernalization at 15 °C was less effective when compared to the temperatures of 5 °C and 10 °C for all three cultivars. Vernalization at 5 and 10 °C of lisianthus seeds cv. Mariachi, Echo and Excalibur led to greater efficiency in the flowering induction and reduction of the crop cycle, important characteristics in the context of large-scale flower production. For cultivating lisianthus as a quality cut flower it is recommended to vernalize seeds of cv. Mariachi and cv. Echo for 24 days at 5 °C and cv. Excalibur for 36 days at 5 °C.

Acknowledgements

To Federal University of Viçosa for the support and CAPES for the grant, to the first author of a PhD scholarship in the Department of Plant Science. To the Sakata Seed Corporation for technical assistance and supply the seed for research.

References

ALVES, C. M. L.; BARBOSA, J. G.; SÁ P. G.; FINGER, F. L.; GROSSI, J. A. S.; MUNIZ, M. A.; CECON, P. R. Efficiency of preservative solutions on the postharvest life/longevity of Lisianthus flowers var. ABC. *Acta Horticulturae*, v. 1060, p. 275-280, 2015.

AMASINO, R. M. Vernalization and flowering time. *Current Opinion in Biotechnology,* London, v. 16, n. 2, p.154-158, 2005.

ARNHOLD, E. *Easyanova*: analysis of variance and other important complementary analyzes. [S.l.: s.n.], 2014.

CAVE, R. L.; JOHNSTON, M. E. Vernalization promotes flowering of a heat tolerant *Calandrinia* while long days replace vernalization for early flowering of *Brunonia*. *Scientia Horticulturae*, Amsterdam, v. 123, n. 3, p. 379-384, 2010.

COOPER, P. I. The absorption of radiation in solar stills. *Solar Energy*, Nedlands, v. 12, n. 3, p. 333-346, 1969.

DOLE, J. M.; WILKINS, H. F. *Eustoma*, *in*: floriculture: principles and species. New Jersey: Pearson Prentice Hall, 2005. 1040 p.

FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA, D. A. *ExpDes*: experimental desings package versão 1.1.2. [S.l.: s.n.], 2013.

FUKUDA, B. Y.; KIYOSHI, F.; OHKAWA, R.; TREATMENT, H. T.; KORENAGA, M. S.; KANEMATSU, K. Classification of *Eustoma grandiflorum* (Raf.) Shinn . cultivars on rosette characteristics ratios. *Journal of the Japanese Society for Horticultural Science,* Japanese, v. 62, n. 4, p. 845-856, 1994.

HA, T. M. A review of plants' flowering physiology: the control of floral induction by juvenility, temperature and photoperiod in annual and ornamental crops. *Asian Journal of Agriculture and Food Sciences*, Thai Nguyen, v. 2, n. 3, p. 186-195, 2014.

HA, T. M.; JOHNSTON, M. E. The effect of low temperature on flowering of *Rhodanthe Floribunda*. *Asian Journal of Agriculture and Food Sciences,* Thai Nguyen, v. 1, n. 5, p. 205-209, 2013.

HAMO, M. L.; VILLACORTA, C.; ZACCAI, M. Characterization of expressed sequence tags from *Lilium longiflorum* in vernalized and non-vernalized bulbs. *Journal of Plant Physiology*, v. 173, p. 72-81, 2015.

HARBAUGH, B. K. Flowering of *Eustoma grandiflorum* (Raf.) shinn. cultivars influenced by photoperiod and temperature. *HortScience*, Alexandria, v. 30, n. 7, p. 1375-1377, 1995.

HARBAUGH, B. K.; ROH, M. S. Rosetting of lisianthus cultivars exposed to high temperature. *HortScience*, Alexandria, v. 27, n. 8, p. 885-887, 1992.

HARBAUGH, B. K.; SCOTT, J. W. Florida Pink and Florida Light Blue-Semi-dwarf Heat-tolerant cultivars of Lisianthus. *HortScience*, Alexandria, v. 34, n. 2, p. 364-365, 1999.

KAGEYAMA, Y.; FUKUSHIMA, Y.; KONISHI, K. Effects of raising seedling at cool temperature and chilling treatment of seed on rosette formation of *Eustoma*. *Journal of the Japanese Society for Horticultural Science*, v. 59, p. 496-497, 1990.

KIM, D. H.; DOYLE, M. R.; SUNG, S.; AMASINO, R. M. Vernalization: winter and the timing of flowering in plants. *Annual Review of Cell and Developmental Biology Journal*, Texas, v. 25, n. 1, p. 277-299, 2009.

KOBORI, M. *Produção de lisianthus de corte.* Atibaia: Sakata, 2012.

LORENZI, H.; SOUZA, H. M. *Eustoma grandiflorum* (Raf.) Shinners. In: LORENZI, H.; MOREIRA de SOUZA, H. (Org.). *Ornamental plants in Brazil*. São Paulo: Plantarum, 2008. p. 615.

OKA, M.; TASAKA, Y.; IWABUCHI, M.; MINO, M. Elevated sensitivity to gibberellin by vernalization in the vegetative rosette plants of *Eustoma grandiflorum* and *Arabidopsis thaliana*. *Plant Science,* Bibao, v. 160, n. 6, p. 1237-1245, 2001.

PERGOLA, G.; OGGIANO, N.; CURIR, P. Effects of seeds and seedlings temperature conditioning on planting, bolting and flowering in *Eustoma russellianum*. *Acta Horticulturae*, v. 314, p. 173-177, 1992.

R CORE TEAM - R. Software R: a language and environment for statistical computing. Versão 3.0.3., 2014.

ROH, M.; HALEVY, A. H.; WILKINS, H. F. *Eustoma grandiflorum*. In: HALEVY, A. H. (Org.). *The handbook of flowering*. Florida: CRC Press, 1989. p. 322-327.

SAKATA. Lisianthus (*Eustoma grandiflorum*) plant production. Yokohama: [s.n.], 2012. n. 1.

______. SAKATA. Sakata's reliable seeds - Flower seed catalogue: (s/n) Yokohama - Japan. 2010.

SANTOS, P. G. M. dos; MOTA, F. W.; VIEIRA, B. C. J.; MOTA FILHO, G. J. V.; MADUREIRA, P. R. Vernalization and cut of apical third of the bulbs in yield and quality of seeds of onion. *Semina: Ciências Agrárias*, Londrina, v. 33, n. 3, p. 989-996, 2012.

TAIZ, L.; ZEIGER, E. *Vegetal physiology*. New Jersey: Artmed, 2013. 782 p.

TANIGAWA, T.; KUROYANAGI, N.; KUNITAKE, T. Effects of low temperature treatment of imbibed seeds of *Eustoma grandiflorum* (Raf.) Shinn. on their germination and subsequent bolting. *Journal of the Japanese Society for Horticulturall,* Kyoto, v. 71, n. 5, p. 697-701, 2002.

XI-HENG, Z.; JIN-CAI, LI; YASUYO, N. Effects of low temperature vernalization treatment of seeds of lisianthus on bolting. *Acta Agriculturae Scandinavica Boreali-Sinica,* Tianjin, v. 26, n. 3, p. 124-127, 2011.