

Dry storage at two temperatures and three lengths of Polo rose stems

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Abstract

Low temperature and hydration of flower stems is associated with increased vase life. Stem length is linked to differences in absorption rate related to anatomical characteristics of vascular ducts. This study aimed to evaluate the effect of two temperatures (4 and 22 °C) and three flower stem lengths (0, 2 and 20 cm) on the postharvest life of Polo roses. In August 2018, Polo rose stems wrapped in kraft paper and plastic bags were divided into two batches that were stored for four days at 22 °C and 4 °C and then subdivided into three groups that were trimmed to 0, 2 and 20 cm in the basipetal direction and placed in a tap water solution to evaluate the vase life. An anatomical description of the three stem lengths was made to determine the xylem vessel diameter, vulnerability and hydraulic conductivity. The results showed that both the application of 4 and 22 °C resulted in a lower rate of water absorption throughout the trial. The flower opening did not differ in 10 of 13 d but was lower with 0 cm stems, with a vase life greater than three days in the 4 °C treatment compared to 2 and 20 cm stems. A lower potential susceptibility to cavitation was determined in 0 cm stems, with vessel elements of 8.65 µm diameter compared to 25.17 µm in longer stems.

Keywords:

absorption rate, dry management, flower stem anatomy, xylem vulnerability.



Introduction

The flow of water in cut flowers occurs in vessels of different sizes; those with smaller diameter are found at the apex of the flower stem (Arriaga *et al.*, 2016; Arriaga *et al.*, 2020). If cavitation occurs at this site, the dissolution of the air bubble would be very rapid and hydration would occur in less time (Van Ieperen *et al.*, 2002; van Doorn, 2012).

In this regard, Arriaga *et al.* (2016) reported that, in Polo roses with 25 cm stems, the diameters and areas of the vessels were 4.41 and 8.4 μm , respectively, which improves water flow and reduces the risk of cavitation. In *Alstroemeria* cv. Rebecca, the diameter of the vessels at 2 cm from the inflorescence was 15.11 μm . Differences in the diameter of the vessels could influence the continuity of the water column, which would affect the hydration of the flower stem (Rosas-Balderas, 2018).

Dry management creates a modified atmosphere that decreases transpiration and maintains the hydration of flower stems for longer, similar to refrigerated storage as CO_2 concentration increases and O_2 concentration decreases (Rudnicki *et al.*, 1986; Aros *et al.*, 2017; Poonsri, 2021). Macnish *et al.* (2009) show that Charlotte rose stems managed dry and cold presented weight losses of 4.8% and a rapid restoration of water conductivity. Cevallos and Reid (2001) report that Ambiance roses with dry management and storage at 10 °C decrease their loss of turgor during vase life. Mosqueda *et al.* (2012) mention that rose flower stems stored at 4 °C at 10 d of vase life had an absorption rate of 33.5 ml.

Authors such as Moraes *et al.* (2017) indicate that, at 1 °C, the flower stems of rose cv. Avalanche can be stored for up to 21 days with no effect on their vase life. However, differences in flower stem lengths can lead to distinct vase life due to variation in vessel diameter.

In Polo roses, research has been carried out analyzing the relationship of vase life with the anatomy in different lengths of the flower stem. Nonetheless, no studies have been carried out that describe this relationship at the base of the flower bud, the pedicel, or very close to the pedicel-flower stem intersection. Thus, this study aimed to evaluate the effect of two storage temperatures (4 and 22 °C) and anatomy associated with three lengths of the flower stem (0, 2 and 20 cm in the basipetal direction) on the absorption rate and postharvest life of Polo rose.

Materials and methods

In August 2018, in a greenhouse of the College of Postgraduates, Montecillo *Campus*, 42 flower stems of Polo roses were harvested and transported dry to the FES-Iztacala laboratory of the National Autonomous University of Mexico (UNAM, for its acronym in Spanish). They were divided into two batches of twenty-one stems each and wrapped in kraft paper and black polyethylene bags; the first batch was stored for four days at 22 ± 3 °C and the second batch was kept at 4 ± 2 °C. They were then divided into three subgroups with seven stems in each. After this period, the flower stems were trimmed and separated by treatments: T1, 0 cm (base of the flower bud), T2, 2 cm and T3, 20 cm in the basipetal direction; subsequently, they were weighed on a Velab[®] ES-1000H scale with an accuracy of 0.01 g. The experimental units consisted of a flower stem placed in a vase with 70 or 300 ml of tap water, van Meeteren *et al.* (2001), which were randomly distributed in a laboratory room (RH, 45%; temperature, 22 °C).

For the anatomy, samples of 3 cm in length were taken from three flower stems of each length and the paraffin inclusion microtechnique was performed (Ruzin, 1999). The following were evaluated.

Fresh weight and solution absorption rate (SAR)

The change in weight of the flower stems was recorded daily; the solution absorption rate was obtained with the equation proposed by Rezvanypour and Osfoori (2011).



Flower opening and vase life

They were obtained by measuring at the top of each flower bud with a Scala[®] digital vernier, 0.01 mm accuracy, and by counting the number of days that the flower buds remained without showing symptoms of senescence, such as wilting, petal drop, pedicel bending or leaf yellowing.

Anatomical description for each length of the flower stems

It was performed with a Leica DM 750 optical microscope; the images were processed with the free software of ImageJ[®] v1.52e (<https://imagej.net/ij/>, 2018).

Number, diameter, distribution and frequency of vessel elements

The number of vessel elements was directly counted in ten 1 mm² fields; the diameter was obtained with the free software of ImageJ[®] v1.52e and with a 0.01 mm calibration micrometer.

Vulnerability index (VI) and relative water conductivity (RWC)

They were obtained with the equations:

$$VI = \frac{\text{Vessel diameter } (\mu\text{m})}{\text{Number of vessels per mm}^2}$$

Carlquist (1977) and $WRC = \text{Vessel radius}^4 \times \text{number of vessels per mm}^2$ (Fahn *et al.*, 1986).

Statistical analysis

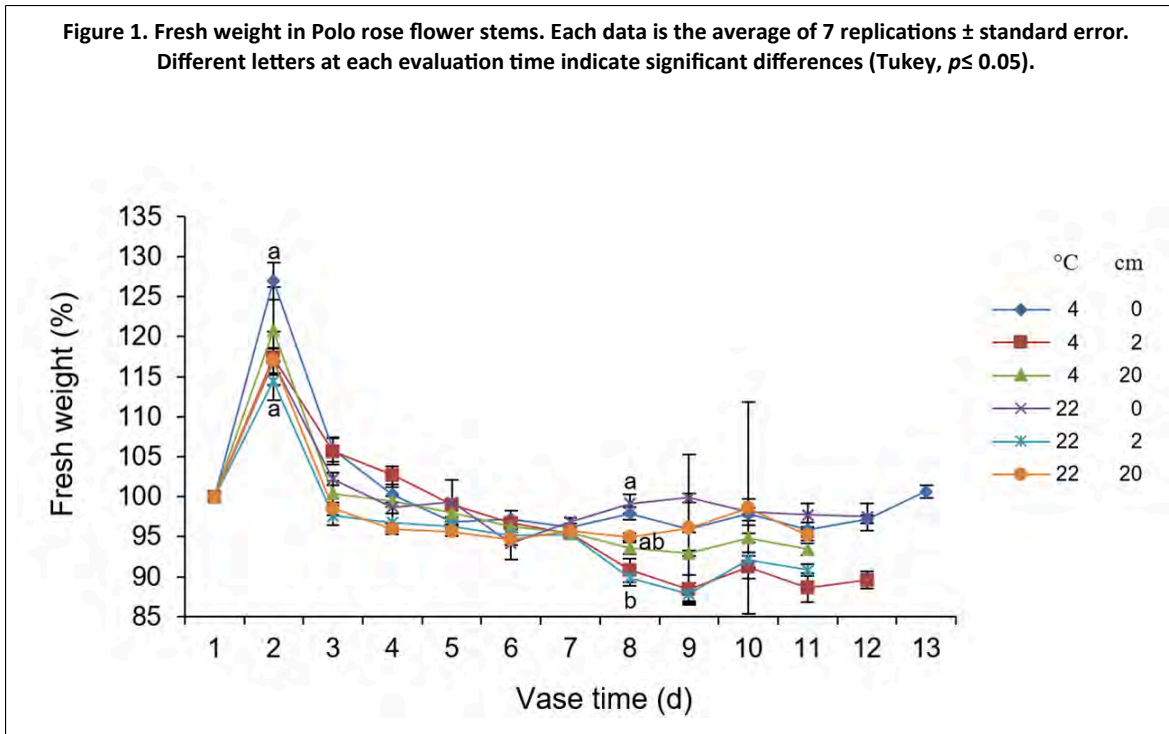
The data were processed with an analysis of variance and mean comparison tests (Tukey, $p \leq 0.05$) using the Statistical Analysis System (SAS[®]) v.9.0 software for Windows (<https://www.sas.com/en-us/software/stat.html>, 2002).

Results and discussion

Fresh weight (FW)

From day 1 to 7, the change in fresh weight had no differences between treatments (p -value 0.05) (Figure 1).





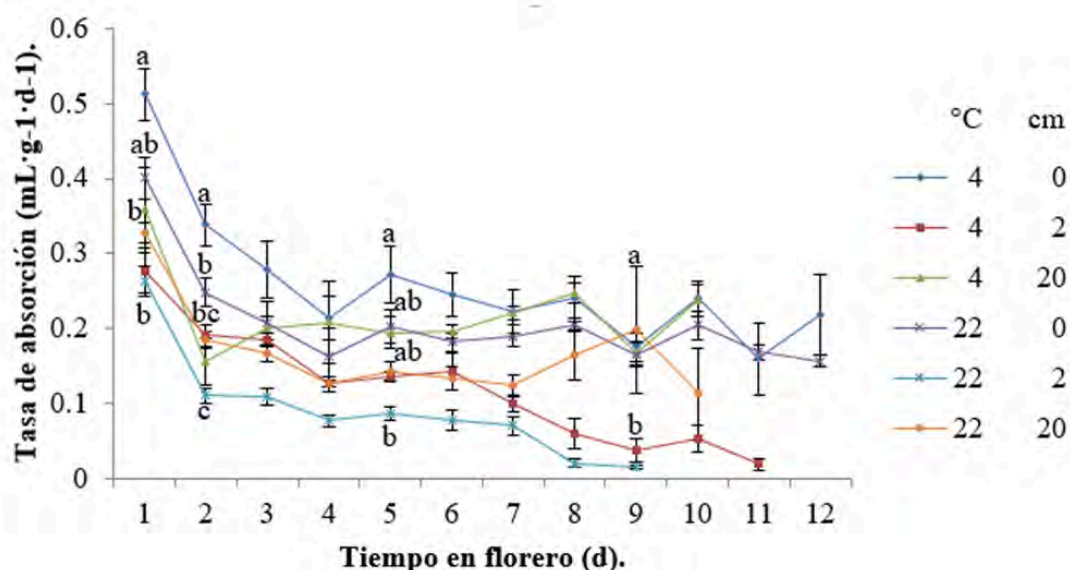
The fresh weight records of Polo roses were similar to those reported by Juárez *et al.* (2008), where when comparing Black Magic roses at 2.5 °C and room temperature, they did not show differences in fresh weight until the seventh day. The transpiration of the flower bud plays a determining role in the demand for water flow through the vascular ducts. The flower, by not having stomata, regulates its hydration according to variations in temperature and relative humidity, and the area of exposure of the petals is modified according to the opening of the flower (Juárez-López *et al.*, 2011).

Solution absorption rate (SAR)

The SAR had differences (p -value ≤ 0.05) throughout the vase life, mainly in stems 2 cm long stored for 4 d at 4 °C and at 22 °C, as the SAR decreased markedly compared to stems 0 or 20 cm long, probably due to differences in the diameter of the vessels (Figure 2).



Figure 2. Solution absorption rate in Polo rose flower stems. Each data is the average of 7 replications \pm standard error. Different letters at each evaluation time indicate significant differences (Tukey, $p \leq 0.05$).



When the stems are placed in the vase, the absorption rate increases in the first few days and then decreases, the reduction in water consumption can be attributed to cavitation events, the later the cavitation occurs the longer the stems will last in the vase (Arévalo *et al.*, 2012; van Doorn, 2012). The lower variation in SAR in 2 cm stems was attributed to the lower risk of cavitation. Rosas-Balderas (2018) mentions that, in alstroemeria cv. Rebecca, 2 cm long stems have vessels with a smaller diameter at this site; the SAR was higher compared to stems 20, 40 and 60 cm long, which would have a higher probability of cavitation. Similar results were obtained in this experiment.

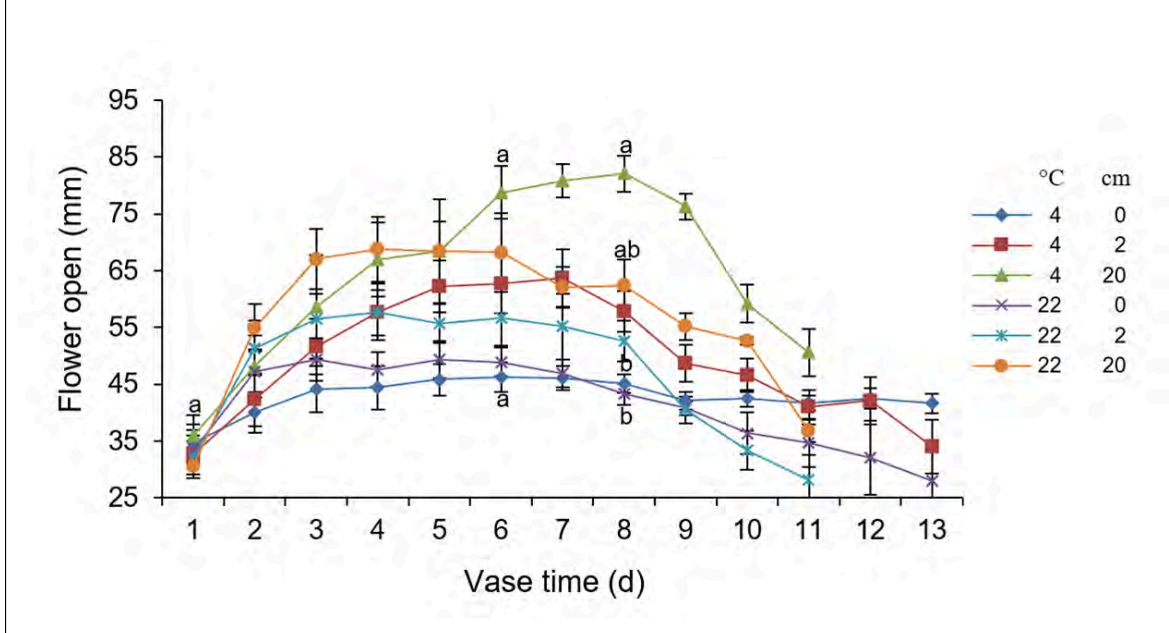
Flower opening (FO)

In Polo roses, storage at 22 or 4 °C did not influence the opening of the flower buds on ten out of thirteen days; this coincides with what was recorded by De la Cruz *et al.* (2018), who report that, in stems of *R. hybrida* Topaz stored dry for 1 to 7 days, there is no difference in the diameters of flower opening. The 20 cm stems were statistically different from the 0 cm stems, with no differences from the other treatments.

In general, the flower opening was grouped according to stem length, ie. 0 cm (4 and 22 °C, 8 d), 2 cm (4 and 22 °C, 7 d), and 20 cm (4 and 22 °C, 7 d). The maximum values of opening obtained at 4 °C were 82.07 mm, 62.62 mm, and 46.9 mm for 20, 2 and 0 cm long stems, respectively, where the latter presented the least variability in this variable throughout the vase life (Figure 3).



Figure 3. Flower opening in Polo rose stems. Each data is the average of 7 replications \pm standard error. Different letters at each evaluation time indicate significant differences (Tukey, $p \leq 0.05$).



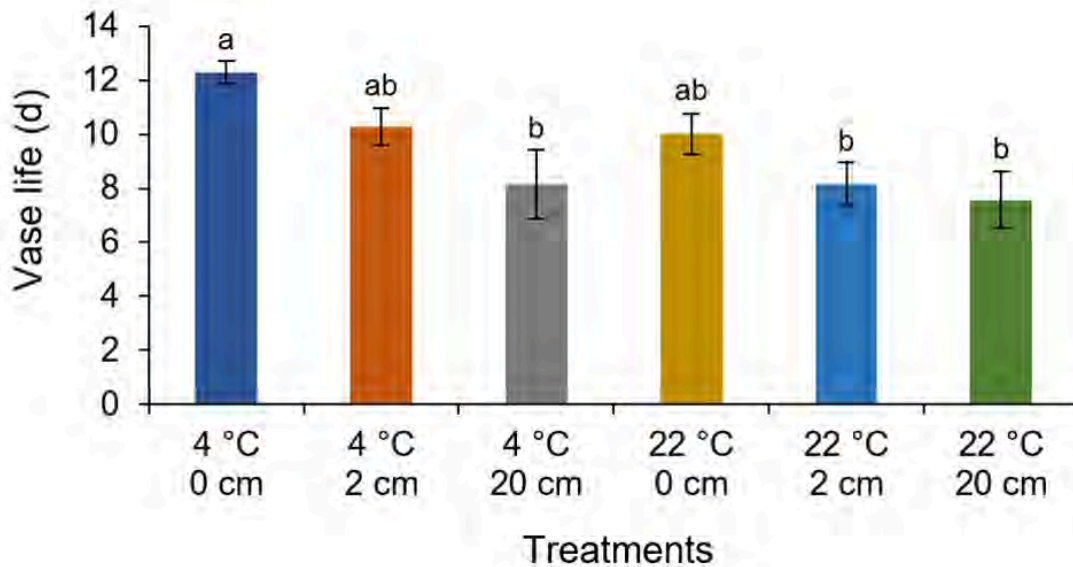
This flower opening could be due to the fact that the main sources of energy for the flower opening are mainly stored in the stems and their reduction (0 and 2 cm) make the nutritional sources decrease and therefore, the flower opening is lower compared to long stems, as suggested by Mosqueda *et al.* (2011).

Vase life (VL)

For this variable, there was a positive effect of cold (4 °C) and stem length (0 cm) on vase life; according to Juárez *et al.* (2008), Grand Gala roses with refrigerated storage extend their vase life by up to 4 d, with an average of 13 d of vase life, which is consistent with the present work since cold stems increased their vase life by 3.2 d on average. Vase life showed significant differences (p -value ≤ 0.05), mainly from the treatments of 0 cm stems, which had an average of 3 days more VL, compared to 20 and 2 cm stems (Figure 4).



Figure 4. Vase time in Polo rose flower stems. Each data is the average of 7 replications \pm standard error. Different letters in each treatment indicate significant differences (Tukey, $\alpha \leq 0.05$).



According to Carlquist (1988), the conduction of water through numerous vessels with a narrow diameter ($\leq 100 \mu\text{m}$) is more efficient than through a few vessels with a wide diameter ($\leq 100 \mu\text{m}$). In this way, the cavitation of narrow vessels is related to a lower loss of conduction capacity. In this study, 0 cm stems with smaller diameter vessels ($20 \mu\text{m}$), being less susceptible to cavitation, may explain the longer vase life. In this experiment, it was considered that the effect of refrigeration added to the improvement in the response.

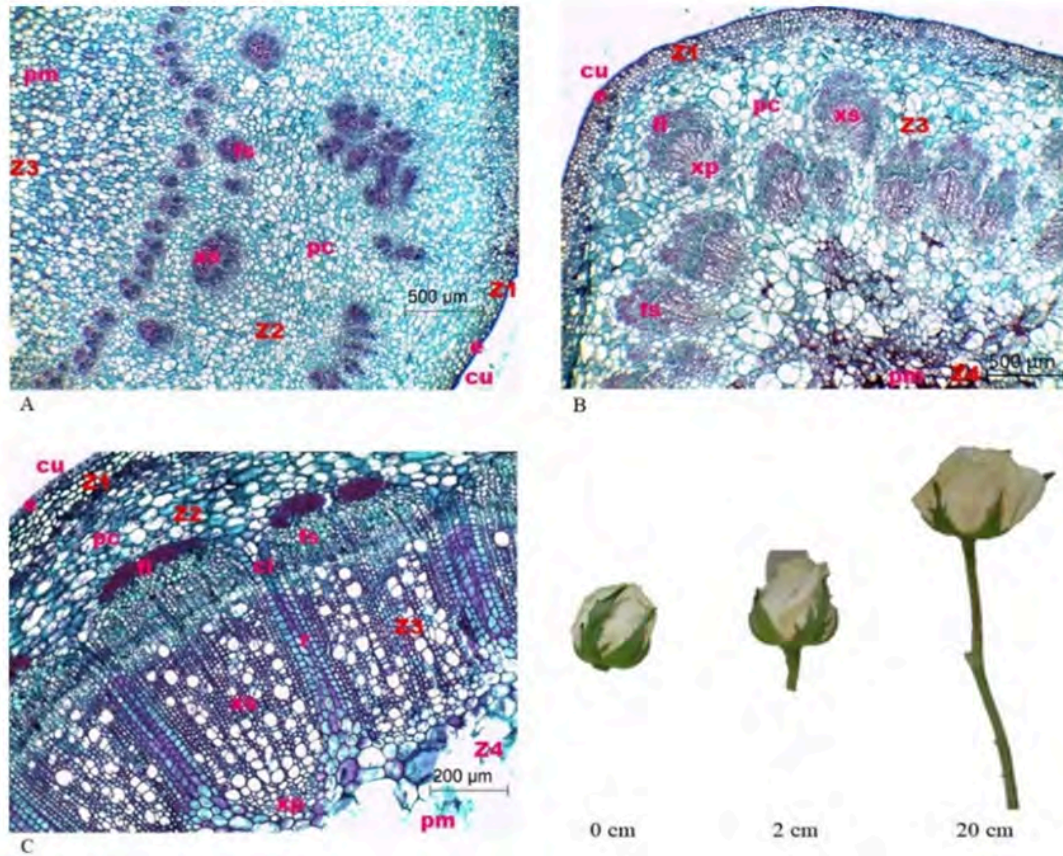
Anatomical description of Polo rose flower stems 0, 2 and 20 cm long

The cross-sections were divided into four zones, the first consisting of the outer wall and a thick cuticle followed by a monostratified epidermis without trichomes. Hernández *et al.* (2009), in Grand Gala and Vega, report the same anatomical features and indicate that water loss is related to the variation in cuticle thickness, which is affected by environmental conditions.

Zone 2 has a continuous band of parenchyma. Zone 3 is represented by vascular bundles. Zone 4 corresponds to the central portion of the stem. In zones 1, 3 and 4 of the 0 cm stems of Polo rose, there was a greater number of vascular bundles, grouped in a compact way and arranged in a radial shape (Figure 5A), it was associated with improved water flow. These characteristics are consistent with what was reported by Cohen *et al.* (2012), regarding the fact that elements with a smaller size of the vessel elements are less susceptible to cavitation.



Figure 5. Anatomy of cuts of Polo rose stems A) 0 cm; B) 2 cm and C) 20 cm. cu= cuticle; e= monostratified epidermis; pc= cortical parenchyma; fi= fibers; ci= interfascicular cambium; fs= secondary phloem; r= radius; xs= secondary xylem; xp= primary xylem; pm= medullary parenchyma. Similar results were obtained in Grand Gala roses, as reported by Hernández *et al.* (2009).



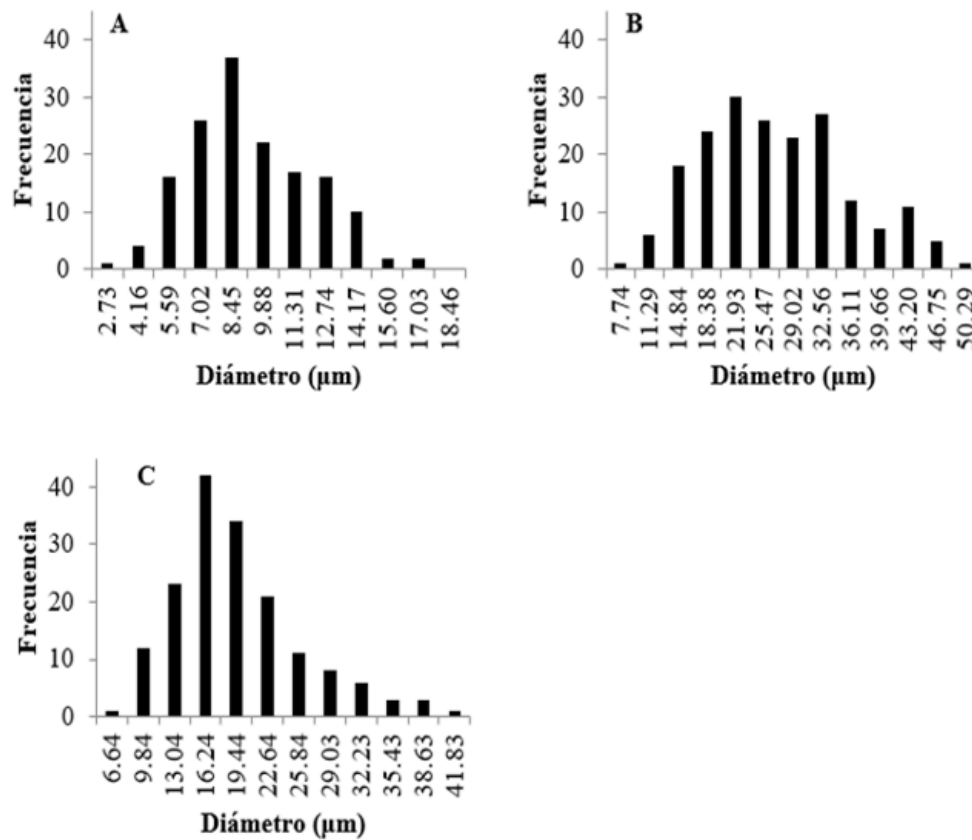
In the 2 cm group, the distribution of vascular bundles showed a significant increase in clusters, where only zones 1, 3 and 4 prevail (Figure 5B). Rosas-Balderas (2018) indicates that, in 2 cm stems of *Alstroemeria* Rebecca, the vascular bundles are more contiguous, more numerous and with a reduced diameter compared to 20 cm stems, while cells are larger towards the center the parenchyma.

The 20 cm stems had a monostratified epidermis, parenchymal cortex, complete vascular cylinder, and parenchymal medulla. In zone 1, the cortex is made up of 6 to 8 layers of parenchymal cells; zone 2 is composed of 11 to 12 parenchymal cell strata with differential thickenings. In zone 3, thick-walled fibers are observed. Vessel elements usually appear isolated in multiserial radial groups with up to 7 cells (Figure 5C).

Frequency distribution

In the basal zone (0 cm), their diameters ranged from 2.7 µm to 17 µm (Figure 6A), with 94.51% between 5 and 14 µm (9 µm range). In the contiguous zone (2 cm), diameters ranged from 7.7 to 50.2 µm (Figure 6B), with 86.14% in the 9-25 µm range (16 µm range). In the xylem of the middle stem zone (20 cm), the diameter of the vessels fluctuated between 6.6 µm and 41.8 µm, with 83.33% concentrating between 14 and 36 µm (22 µm range) (Figure 6C).

Figure 6. Distribution of vessel elements per mm^2 . A) 0 cm; B) 2 cm and C) 20 cm from the flower bud.



The dispersion in the diameter of vessels measured by the interval with the highest percentage referred to above decreased inversely to the length of the stem. Cohen *et al.* (2012) report that the rose cultivars Lovely Red and Rouge Baiser presented 267 and 308 vessels per mm^2 and vessel area of 0.33 and 0.21 mm^2 , respectively, which means that Rouge Baiser is a cultivar with greater aptitude for water transport, but, at the same time, more susceptible to wilting due to lack of water.

In their study, Arriaga *et al.* (2016) mention the relationship between stem lengths of 25, 35, and 50 cm with their vessel diameter of 4.41, 12.4, and 12.51 μm , respectively, and indicate a direct relationship between this variable and the absorption rate and vase life. Hernández *et al.* (2009) report that the xylem arrangement in two cultivars of *R. hybrida* is diffuse porous, that is, rings are not distinguished, and regarding the diameter of the vessels, it was between 13.2 μm and 39.3 μm in Grand Gala and from 14.1 μm to 67.7 μm in Vega.

The degree of dispersion of the diameters of xylem vessels together with their decrease as their approach the point of insertion of the flower bud is evidence of a lower probability of cavitation in the vicinity of the flower head, that is, of an anatomical feature that protects or favors the supply of water to the flower. This variation was shown with a wide spectrum of vessel diameters, where a larger diameter contributes to a greater extent to cavitation and loss of water conductivity (Arriaga *et al.*, 2016).

Number of vessels, vulnerability index (VI), and relative water conductivity (RWC)

The potential water conduction capacity is a function of their number per unit area because it is determined by the dimensions of the xylem vessels. De la Cruz *et al.* (2016) report, for the same species with different cultivars, an average of 388.7 vessels per mm² for 54 cm stems and 242.6 vessels per mm² for stems 33 cm long, that is, a decrease in the acropetal direction, coinciding with what was obtained for the Polo rose of the present study (180, 20 cm; 144, 2 cm; 132, 0 cm). In both cases, there was a decrease in the average number of vessels the closer they were to the flower.

Such variation determines the susceptibility to obstruction of the flow of water, where the vulnerability index indicates that the closer it is to the value of 1, the more vulnerable it will be to the interruption of the flow of water. In the present study, the VIs of the middle zone (20 cm), contiguous zone (2 cm), and at the base of the flower bud (0 cm) were 0.17, 0.1, and 0.06, respectively; that is, from greater to lesser degree of vulnerability in the far and near portions of the flower bud. With the RWC, a gradient from higher to lower xylem transport potential is observed between the distal and proximal portions to the base of the flower bud (Table 1).

Table 1. Number of vessels, diameter of elements, vulnerability index, and relative water conductivity in cross-sections of Polo rose flower stems 20, 2 and 0 cm long.

Stem length (cm)	Num. of vessels	Vulnerability index	Relative water conductivity
0	132.33 a ^y	0.06 c	72970.67 c
2	144 a	0.11 b	2396013.59 b
20	180.33 a	0.16 a	8746532.75 a
HSD	94.43	0.04	1380000
CV	24.76	15.46	14.71

^y = different letters in each column indicate significant differences (Tukey, $\alpha \leq 0.05$); each data is the average of three 1 mm² quadrants. HSD= honest significant difference; CV= coefficient of variation.

Arriaga *et al.* (2016) results show, for Polo, that the VI varies depending on the length of the flower stem, as there were VIs of 0.14, 0.83, and 1.29 for Polo rose flower stems 25, 35, and 50 cm long, respectively. The highest susceptibility (VI) of Polo roses to water stress occurred in the 20 and 2 cm stems.

The RWC, although higher at 20 cm, decreases when embolism occurs; on the other hand, in 0 cm stems with smaller diameter vessels, embolism and RWC decrease, but with a constant value, which results in better hydration of the flower bud. According to Carlquist (1988), conduction through many vessels with a narrow diameter is more efficient than with few vessels with a large diameter. Thus, the cavitation of narrow vessels causes a lower loss of conduction capacity, which was consistent with the anatomy of the 0 cm stems.

Nevertheless, the presence of numerous vessels of smaller diameters (#25 μ m) increases the total conductivity of water, a characteristic that extended the vase life by an average of 03 days compared to stems 2 and 20 cm long. Ahmad *et al.* (2012) report that, even with periods of two weeks in dry, Angelique and Kardinal roses weight less compared to the stems kept wet; it is concluded that dry management results in better water relations and that wet management does not necessarily prevent excessive loss of water in roses.

The dimensions of the vessels in 0 cm stems contrast with those of the 2 and 20 cm stems, where hydration depends on the distance to be traveled to the flower. Although the flow of water varies depending on the diameter of xylem vessels, it decreases when the water reaches the base of the flower head due to the reduction in size of the xylem vessels. RWC and vessel vulnerability do not necessarily translate into predictable results when evaluating physiological variables due to the absence of differences in fresh weight and solution absorption rate for Polo roses.

It is possible to state that the distance between the vulnerability potential and physiological variables, such as SAR, weight loss, or evapotranspiration, is influenced by the amplitude of the vessel diameter range associated with the distance from the base of the stem to the flower bud. The analysis of the dispersion of diameters and grouping of vessels is informative of the regulation of the effectiveness of flower hydration.

Conclusions

There were no differences in the weight of flower stems pre-treated with 0 and 22 °C. The absorption rate did not show a definite pattern of differences between stem lengths, where the greatest fluctuations were observed in stems 0 and 20 cm long. The flower opening was lower with the stem length of 0 cm, but it had a vase life three days longer compared to the lengths of 2 and 20 cm. In 0 cm flower stems, storage at 4 °C was associated with an increase in vase life.

The flow of water through the vascular ducts presents contrasting diameters in each stem length, and their influence on water absorption and flower weight did not show a defined pattern. The diameter of xylem vessels decreases acropetally. Smaller diameter vessels have lower potential vulnerability and water conductivity to cavitation and viceversa.

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Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 December 2024
Date accepted: 01 March 2025
Publication date: 27 March 2025
Publication date: Feb-Mar 2025
Volume: 16r
Issue: 1
Electronic Location Identifier: e3367
DOI: 10.29312/remexca.v16i2.3367

Categories

Subject: Articles

Keywords:

Keywords:

absorption rate
dry management
flower stem anatomy
xylem vulnerability

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