KSÜ Tarım ve Doğa Derg 27 (Ek Sayı 2), 361-371, 2024
KSU J. Agric Nat 27 (Suppl 2), 361-371, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1434326

Effects of Some Bacterial Isolates on Vase Life of Cut Rose Flowers

Deniz TİRYAKİ[1], Fazilet PARLAKOVA KARAGÖZ[2]✉, Ökkeş ATICI[3], Atilla DURSUN[4]
[1,3]Department of Biology, Faculty of Science, Atatürk University, 25240 Erzurum, Turkey, [2,4]Department of Horticulture, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Turkey
[1]https://orcid.org/0000-0002-7452-9236, [2]https://orcid.org/0000-0001-7417-1716, [3]https://orcid.org/0000-0001-6256-5101, [4]https://orcid.org/0000-0002-8475-8534
✉: f.parlakova@atauni.edu.tr

ABSTRACT
To reduce the impact of rapid temperature changes during transportation on cut flowers, continuous cooling should be provided throughout the process. The frigid transportation conditions required to transport cut flowers from their production facilities to the point where they will be marketed are quite expensive. Preliminary applications that can significantly reduce this cost should be made. One of these applications is the use of symbiotic psychrotolerant bacteria, which thrive at low temperatures, to improve the transportation and shelf life of cut flowers. Cut roses are commercially transported and stored at +2-4°C. In this study, the application of psychrotolerant bacteria isolated from plants aims to investigate their effects on the transportation and vase life of cut roses under unfavorable transport temperature conditions (+10 °C), aiming to minimize potential quality losses. For this objective, 18 bacterial strains were isolated from the leaf apoplasts of cold-resistant wild plants. These strains, which exhibit cold tolerance and the ability to block ethylene synthesis in plants, were used both separately and in combination. Bacterial solutions were applied to cut rose leaves (*Rosa hybrida* L. cv. Samourai), and all treatment groups were kept in controlled conditions at +10°C for 9 days. Control 1 and Control 2 were treatments in which only pure water was sprayed on the green leaves of cut roses at temperatures of +2 °C and +10 °C, respectively. At the end of the experiment, the cut roses were evaluated according to vase life, fresh weight, visual quality, and cold damage (CD). The longest vase life was determined in D3 (DT-10 isolate-*Bacillus cereus*), D4 (DT-11 isolate-*Bacillus cereus*), M1 (DT-17 isolate-*P. proteolytica*) and K1+B8 (*Bacillus cereus* + *S. kitahiroshimense*) applications. These applications, which have obtained the highest vase lifetimes, and B5, B8, D1, D2, K3, Ç1, Ç2, M2, and D1 + Ç1 applications were in the same statistical group. The D3, D4, M1, and K1+B8 treatments increased the vase life of cut roses by 55.55% compared to Control 2. Control 1 and D1+Ç1 treatments showed the highest relative fresh weight increase in 66.06% and 64.28%, respectively. Among the groups, the D2 (DT-6 isolate-*Bacillus cereus*) had the greatest visual quality score. The lowest CD% was observed in the application involving the D2 isolate, correlating with the highest visual quality determined under the same D2 application. The study revealed that postharvest application of *Bacillus cereus* isolates (especially DT-6 and DT-10) to cut roses increased their vase life.

Horticulture

Research Article

Article History
Received : 12.02.2024
Accepted : 26.09.2024

Keywords
Apoplast
Psychrophile bacteria
Vase life
Cut flower
Transport

Bazı Bakteriyel İzolatların Kesme Gül Çiçeklerinin Vazo Ömrüne Etkileri

ÖZET
Kesme çiçeklerin, taşıma sırasındaki ani sıcaklık değişimlerinden daha az etkilenmeleri için taşıma sürecinde kesintisiz soğutma yapılması gerekmektedir. Kesme çiçeklerin üretim yerlerinden pazarlanacakları noktaya taşınmasında gerekli olan soğuk taşıma koşulları çok maliyetli olmaktadır. Bu maliyetin azaltılmasında etkili olabilecek ön uygulamalar yapılmalıdır. Bu uygulamalardan biri düşük sıcaklıklarda yaşayabilen psikrotolerant bakterilerin kesme çiçeklerin taşınması ve

Bahçe Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 12.02.2024
Kabul Tarihi : 26.09.2024

sonrasında raf ömrünün uzatılmasında uygulanabilmesidir. Kesme güller ticari olarak +2-4°C'de taşınmakta veya depolanmaktadır. Bu çalışmada, bitkilerden izole edilen psikrotolerant bakterilerin uygulanmasının, kesme güllerin uygun olmayan taşıma sıcaklığı koşullarında (+10 °C) taşınması ve vazo ömrü üzerindeki etkilerini araştırmak ve olası kalite kayıplarını minimuma indirmek amaçlanmıştır. Bunun için soğuğa dayanıklı yabani bitkilerin yaprak apoplastından izole edilen, soğuğa tolerans gösteren ve etilen sentezini inhibe etme yeteneği gösteren 18 bakteri suşu hem ayrı ayrı hem de kombinasyon halinde hazırlanmıştır. Daha sonra kesilmiş kesme güllerin (*Rosa hybrida* L. cv. Samourai) yapraklarına bakteriyel solüsyonlar uygulandı ve tüm uygulama grupları 9 gün boyunca +10°C'de kontrollü koşullar altında tutulmuştur. Kesme güllerin yeşil yapraklarına sadece saf su püskürtülen kontrol grubu uygulamalar olan Kontrol 1 Kontrol 2 uygulaması, sırası ile +2 °C ve +10 °C sıcaklık koşullarında bekletilmişlerdir. Deneme sonunda, kesme güller vazo ömrü, taze ağırlık, görsel kalite ve soğuk hasarına (CD) göre değerlendirildi. En uzun vazo ömrü D3 (DT-10 izolatı- *Bacillus cereus)*, D4 (DT-11 izolatı- *Bacillus cereus)*, M1 (DT-17 izolatı- *P. proteolytica)* ve K1+B8 (*Bacillus cereus + S. kitahiroshimense)* uygulamalarında belirlendi. En yüksek vazo ömrü elde edilen bu uygulamalar ile B5, B8, D1, D2, K3, Ç1, Ç2, M2 ve D1+Ç1 (karışık) uygulamaları aynı istatistiksel grupta yer almıştır. D3, D4, M1 ve K1+B8 uygulamaları Kontrol 2 ile karşılaştırıldığında kesme güllerin vazo ömrünü %55,55 oranında arttırmıştır. En yüksek bağıl taze ağırlık artışı Kontrol 1 (%66,06) ve D1+Ç1 (%64,28) uygulamalarında tespit edildi. Gruplar arasında en yüksek görsel kalite puanını bakteriyel uygulama D2 (DT-6 isolate- *Bacillus cereus)* almıştır. En düşük CD% değeri D2 bakteri izolatının kullanıldığı uygulamada belirlenmiştir. En iyi görsel kalite puanı da minimum CD%'nin gözlemlendiği D2 uygulamasında saptanmıştır. Çalışmada, kesme güllere hasat sonrası *Bacillus cereus* izolatları (özellikle DT-6 ve DT-10) uygulanmasının, vazo ömrünü artırdığı ortaya koyulmuştur.

Anahtar Kelimeler
Apoplast
Psikrofil bakteri
Vazo ömrü
Kesme çiçek
Taşıma

Atıf Şekli: Tiryaki, D., Parlakova Karagöz, F., Atici, Ö., & Dursun, A., (2024). Bazı Bakteriyel İzolatların Kesme Gül Çiçeklerinin Vazo Ömrüne Etkileri. *KSÜ Tarım ve Doğa Derg 27* (Ek Sayı 2), 361-371. https://doi.org/10.18016/ksutarimdoga.vi.1434326

To Cite : Tiryaki, D., Parlakova Karagöz, F., Atici, Ö., & Dursun, A., (2024). Effects of Some Bacterial Isolates on Vase Life of Cut Rose Flowers. *KSU J. Agric Nat 27*(Suppl 2), 361-371. https://doi.org/ 10.18016/ksutarimdoga.vi.1434326

INTRODUCTION

Cut flowers, a key commodity in the ornamental plant industry, rank as the most traded group globally. The most crucial factor in facilitating the trade of these products is their ability to be mass-produced and easily transported (Barlas et al., 2019). The prolonged vase and/or shelf life significantly influences the pricing of both cut flowers and potted plants. Disruptions during transportation, a final stage from sowing to consumer reach, can lead to a loss of value or render the flowers less valuable than their production cost. This disturbance also impacts consumer demand and satisfaction with cut flowers (Onozaki et al., 2001). It has been reported that 25% of cut flowers produced in the world are lost during the transportation and storage process (Ünsal, 2022). In cut flower production for domestic consumption in our country, it is estimated that the quality loss rate of cut flowers that occurs during the transportation and storage process is around 30-50%, especially due to the inability to provide a cold chain. However, according to research conducted with exporters, it was reported that the highest cost during cut flower production belongs to transportation costs at 35% (Sönmez, 2012). It has been reported that the average vase life of different types of cut flowers decreases by 32% after 4 days of transportation (Kazaz, 2015).

Studies conducted on various cut flowers have shown that post-harvest lifespan depends on the respiration rate, and flower lifespan decreases with increased respiration. Respiratory speed increases in direct proportion to temperature. For these reasons, the temperature after production or harvest must be reduced to the lowest temperature at which the flower or plant will not be damaged, and it must be ensured that the cold chain is not broken during storage and

transportation throughout all marketing channels. The temperature just above the freezing point, 0°C, is the appropriate storage temperature for many products. Low temperatures can control metabolism, reduce the consumption of stored compounds and the amount of water lost through respiration, and limit the development of pathogens (Da Silva, 2003; Jahnke et al., 2020). Additionally, low temperatures between 0 °C and 2 °C are recommended for storing rose-cut flowers (Jahnke et al., 2022). Despite all these justifications and requirements, cut flowers and potted plants are generally stored or transported at high temperatures in various marketing channels. This situation reduces quality by increasing water loss and accelerating metabolism and ultimately causes damage to everyone, from the flower producer to the consumer. Uninterrupted cooling is required for cut flowers during the transportation process so that they are less affected by sudden temperature changes during transportation (Çelikel, 2020). The cold transport conditions required to move cut flowers from production areas to market points are very costly and often overlooked. To effectively reduce these costs, preventive measures that are often deemed necessary are implemented. One of these measures is the use of psychrophilic (cold-loving) bacteria in the transport of cut flowers. These bacteria thrive at low temperatures (-5 to 15 °C) and exhibit strong adaptations to tolerate such conditions (Arda, 2000). Some psychrophilic microorganisms were isolated from various environments such as seas, oceans, soil, fish, and vegetables (Graumann, 1996). For example, psychrophilic bacteria isolated from subalpine soil in the Northwestern Indian Himalayas have been shown to increase cold tolerance in wheat (Selvakumar et al., 2008).

Additionally, such bacteria have also been shown to exist in apoplastic areas of certain plants. The apoplast is a continuous space in plants formed by inter and extracellular spaces, cell walls, and dead cells (e.g. xylem) that lie outside the cell membranes. For a plant, the apoplast is a dynamic region where most processes such as growth, nutrition, signal perception, and stress response regulation occur (Atici & Nalbantoglu, 1999). Certain psychrophilic bacteria living symbiotically in the apoplast of some plants can significantly contribute to the plant's abiotic stress tolerance. For example, Tiryaki et al. (2019) demonstrated the success of psychrophilic bacteria isolated from the leaf apoplasts of 14 cold-tolerant wild plants and 2 different crops in increasing cold stress tolerance in beans (*Phaseolus vulgaris* L.). On the other hand, studies have reported that bacteria extend vase life, depending on the type of bacteria present in the vase solution (van Doorn et al. 1991; Jacob & Kim, 2010; Carlson et al., 2015). For instance, two strains of *Pseudomonas fulva* and *Escherichia coli*, also known as biocontrol bacteria, increased the vase life of cut *Zinnia elegans* (Carlson et al., (2015). Naing et al. (2017) also noted that *Enterobacter cloacae* was able to extend the vase life of cloves by 3 days and played an important role in the biological control of microorganisms causing petal senescence. According to our knowledge, there is no existing research attempting to determine the impact of psychrophilic Plant Growth-Promoting Bacteria (PGPB) isolated from cold-resistant wild plants on the vase life of cut flowers in cut flower transportation.

The cold transportation conditions required to transport cut flowers from their production sites to the point where they will be marketed are very costly. Preliminary applications that can be effective in reducing this cost should be made. One of these applications is that psychrophile (cold-loving) bacteria, which can live at low temperatures and have high adaptations to tolerate this, can be applied to the transportation of cut flowers. Cut roses are commercially transported and stored at +2-4 °C. The main purpose of this research is to examine the effects of treating cut roses with psychrophile bacteria and then transporting them under inappropriate transportation temperature conditions (+10 °C) on the vase life of cut roses and reducing possible quality losses. For this purpose, in our study, the effects of 18 different bacterial isolates with psychrophilic properties isolated from the leaf apoplast (intercellular) of some cold-resistant wild plants were investigated in extending the vase life and reducing quality losses that may occur during the transportation of cut roses. The results of this study are believed to contribute to reducing quality and plant losses during transportation, a crucial issue in the cut flower sector, while also aiding in lowering transportation and storage costs and extending the vase life of cut flowers.

MATERIAL and METHOD

Plant Material

The research was carried out, between April and December 2022, in the climate chamber and application laboratory of the Atatürk University Faculty of Science Department of Biology. The red standard type 'Samourai' cut rose cultivar belonging to the *Rosa hybrida* L. species was used as plant material. According to the catalog data of the breeder company, the petal color of the Samourai variety is red, the flower stem length is 80-90 cm, the vase life is 10-12 days, the bud length is 5.0-6.0 cm, the number of petals is 35-40 (Anonymous, 2022). In the world cut rose trade, the market share of red varieties is 30%. In Turkey, this rate is 80% (Ünsal, 2022). In addition, since the Samourai cultivar is among the most-grown rose varieties in Turkey, it was chosen as the plant material in the research.

Harvesting Cut Flowers

Cut rose flowers were harvested early in the morning from the greenhouse of a company that produces commercial cut roses in Antalya (Turkey) on 11 November 2024, at commercial harvest maturity (as the petals begin to curl back) (Ueyama & Ichimura, 1998). After harvesting, it was soaked in water for 3-4 hours. The cut roses were given to an agreed bus company within the same hours, and they reached us within 22 hours by road. No cooling conditions are provided for bus transportation. It was ensured that the cut roses have as many leaves as possible, their leaves are not cleaned, and they are packaged in a way that will minimize damage during transportation (Figure 1). The experiment was set up by applying the cut flowers as soon as they arrived.

Figure 1. The cut roses were packaged in a way (placed in perforated cardboard boxes) that will minimize damage during transportation

Şekil 1. Kesilen güller taşıma esnasında zarar görmeyecek şekilde (delikli karton kutulara konularak) paketlenmiştir

Preparation of Bacterial Isolates

In order to isolate psychrophilic bacteria, 14 wild and 2 cultivated plants (Table 1) species resistant to cold were utilized, collected from Mount Palandöken in Erzurum Province, Atatürk University campus (day/night temperatures of -1°C/-20°C), and the city center of Erzincan (day/night temperatures of 5°C/-2°C) (Tiryaki et al., 2019). Bacteria were isolated from the leaf apoplasts of these plants and after purification, species identification was made according to 16S rRNA sequence analysis and Vitek technique. Twenty plant growth-regulating bacteria (PGPB) isolates belonging to 10 bacterial species were identified from the leaf apoplast of plants (Table 1). These are *Sphingobacterium faecium* (isolates; DT-1, DT-2, DT-3, DT-9, DT-15), *S. kitahiroshimense* (DT-4), *Staphylococcus intermedius* (DT-5), *Bacillus cereus* (DT-6, DT-10, DT-11, DT-12), *Pseudomonas fragi* (DT-7, DT-8), *P. chloropaphis* (DT-14), *P. fluorescens* (DT-16, DT-18), *P. proteolytica* (DT-17, DT-19), *Raoultella ornithinolytica* (DT-13), *Brevibacterium frigoritolerans* (DT-20) (Tiryaki et al., 2019). These bacterial isolates were freshly cultured one week before the cut roses arrived and were prepared separately in liquid media. The bacterial concentration of each solution was adjusted to 10^8 CFU/ml.

Post-harvest Carrying out Pre-Treatment

The flowers, which were harvested at a length of 70 cm in the producer's greenhouse and brought to the processing house, were packaged and placed in perforated cardboard boxes and brought by road to the laboratory (Erzurum) where the study would be carried out within approximately 22 hours. The bottom parts of the flowers brought to the laboratory were cut at an angle to a length of 2.5 cm.

Application of Bacterial Solutions

Application groups consisted of 18 bacterial isolates and 2 control groups (pure water), each with three repetitions. The solutions from the application groups were sprayed to fully moisten the green leaves of cut roses. Applications were made once to the cut roses. The control-2 received no bacterial application; only pure water was sprayed on the green leaves, and these were placed at +10°C in the dark (Figure 2a-f). Following the completion of the spraying process, the cut roses were enveloped in paper packages and positioned in perforated boxes. These boxes were then situated in a climate chamber adjusted to environmental conditions (+10°C in the dark) within the transport vehicle. Concurrently, another application group was prepared, where roses were kept in the dark, enclosed in paper packages, and placed in perforated boxes at 0°C (Çelikel, 2020), just above the freezing point, with pure water sprayed solely on the leaves (Figure 2a-f). The applications and their corresponding codes are detailed in Table 2. The roses earmarked for application were stored in water. For 5 days, cut roses were kept at both temperatures (+10°C and 0°C). On the fifth day, to initiate the vase life experiment (Figure 2 g-h), the lower portions of the flowers were cut at an oblique angle to a length of 2.5 cm, and any remaining leaves in the vase water were manually removed.

Table 1. Bacteria and the plants from which they were isolated (Tiryaki et al. 2019).
Çizelge 2. Bakteriler ve izole edildikleri bitkiler (Tiryaki ve ark. 2019).

Plant	Bacteria	Isolate	Plant	Bacteria	Isolate
Onosma isauricum	*Sphingobacterium faecium*	DT-1	*Fragaria vesca*	*Bacillus cereus*	DT-11
Verbascum cheiranthhifolium	*Sphingobacterium faecium*	DT-2	*Taraxacum sieheanum*	*Bacillus cereus*	DT-12
Chenopodium botrys	*Sphingobacterium faecium*	DT-3	*Galanthus gracilis*	*Raoultella ornithinolytica*	DT-13
Chenopodium foliousum	*Sphingobacterium kitahiroshimense*	DT-4	*Galanthus gracilis*	*Pseudomonas chloropaphis*	DT-14
Myosotis alpestris sp.	*Staphylococcus intermedius*	DT-5	*Galanthus gracilis*	*Sphingobacterium faecium*	DT-15
Capsella bursa-pastoris sp.	*Bacillus cereus*	DT-6	*Colchicum speciousum*	*Pseudomonas fluorescens*	DT-16
Artemisia austriaca	*Pseudomonas fragi*	DT-7	*Colchicum speciousum*	*Pseudomonas proteolytica*	DT-17
Draba nemorosa	*Pseudomonas fragi*	DT-8	*Scilla siberica*	*Pseudomonas fluorescens*	DT-18
Raphanus raphanistrum	*Sphingobacterium faecium*	DT-9	*Scilla siberica*	*Pseudomonas proteolytica*	DT-19
Trifolium repens	*Bacillus cereus*	DT-10	*Erodium cicutarium*	*Brevibacterium frigoritolerans*	DT-20

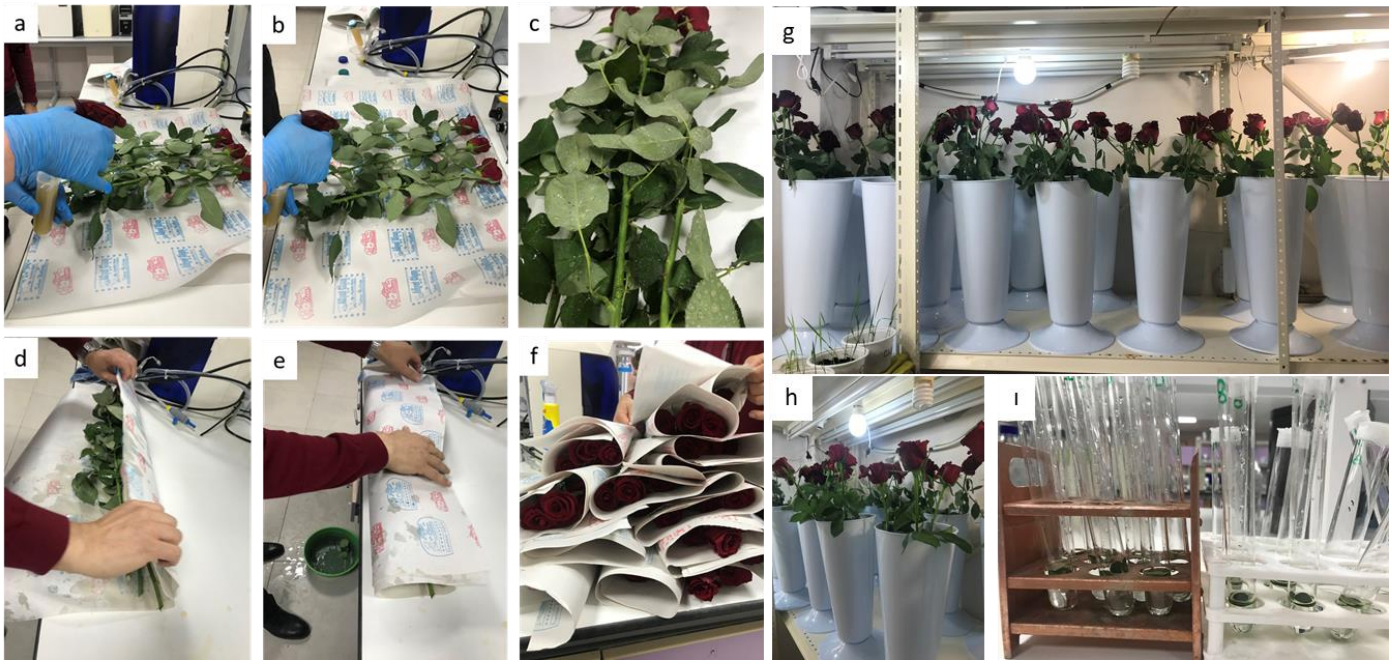

Figure 2. Spraying the leaves of cut roses with application solutions until they are completely wet, then wrapping them in paper again and placing them in a dark environment at +10°C (a-f); roses removed on the 5th day of bacterial treatment and placed in vases in climate chamber conditions (g-h); stage of construction process determining the % cold damage on leaves (ı)

Şekil 2. Kesme güllerin yapraklarına tamamen ıslanacak şekilde uygulama solüsyonları püskürtülerek tekrar kağıda sarılarak +10 °C'de (a-f) karanlık ortama hazırlanması; güller bakteri uygulamalarının 5. gününde çıkarılıp iklim odası koşullarında vazolara yerleştirildi (g-h); yapraklarda soğuktan kaynaklanan zararın %'sini belirleme aşaması (ı)

Conditions for Determining Vase Life

The vase life of flowers was assessed under controlled conditions in a growth chamber with a temperature of 21±2.0°C, relative humidity of 65±5%, light intensity of 1000 lux, and a day length of 12 hours (Ueyama & Ichimura, 1998; Lü et al., 2010). The vases utilized for the vase life studies had a volume of 5 liters and were filled with 3 liters of pure water. Each vase accommodated three cut roses (Figure 2 g-h).

Parameters Examined in Cut Flowers

Vase life (days): Vase life for cut rose flowers are accepted as the number of days from the day the flowers are placed in the vase (start) to the day when the petals begin to fade, and the flower necks begin to bend (Ichimura et al. 1999; Lü et al. 2010).

Table 2. The applications and codes used in the study.
Çizelge 3. Çalışmada yapılan uygulamalar ve kodları.

	Applications	Bacteria	Temperature / Light
1	Control-1		+2 °C Darkness
2	Control-2		10 °C Darkness
3	B1 (DT-1 isolate)	*Sphingobacterium faecium*	10 °C Darkness
4	B3 (DT-2 isolate)		
5	B4(DT-3 isolate)		
6	B5 (DT-9 isolate)		
7	B6 (DT-15 isolate)		
8	B8 (DT-4 isolate)	*S. kitahiroshimense*	10 °C Darkness
9	D1 (DT-5 isolate)	*Staphylococcus intermedius*	
10	D2 (DT-6 isolate)		
11	D3 (DT-10 isolate)	*Bacillus cereus*	10 °C Darkness
12	D4 (DT-11 isolate)		
13	K1 (DT-12 isolate)		
14	K2 (DT-7 isolate)	*Pseudomonas fragi*	10 °C Darkness
15	K3 (DT-8 isolate)		
16	G1 (DT-14 isolate)	*P. chloropaphis*	10 °C Darkness
17	Ç1 (DT-16 isolate)	*P. fluorescens*	10 °C Darkness
18	Ç2 (DT-18 isolate)		
19	M1 (DT-17 isolate)	*P. proteolytica*	10 °C Darkness
20	M2 (DT-19 isolate)		
21	K1+B8 [(DT-12 isolate) + (DT-4 isolate)]	*Bacillus cereus + S. kitahiroshimense*	10 °C Darkness
22	D1+Ç1 [(DT-5 isolate) + (DT-16 isolate]	*Staphylococcus intermedius + P. fluorescens*	

Visual quality: Fading, darkening, bluing, curling backwards, drying and falling off of the petals of all flowers, blooming and bending of the flowers, and yellowing, drying and falling off of the leaves were taken into consideration and evaluated on a 1-5 scale (Score 1: very bad, Score 2: bad, Score 3: medium, Score 4: good, Score 5: very good). Visual quality assessment was made on the 8th day of the flowers' vase life.

Viability Test (Membrane permeability): By measuring cold damage (%) in control and bacteria-applied plant leaves, the role of the applied bacteria in preventing cold damage in the leaves was determined (Figure 2i). The determination of this parameter was carried out as follows: 0.1 g of fresh leaf sample was placed in each of the 20 test tubes. The tubes were placed collectively in a water bath that could be adjusted up to -16°C (alcohol mixed with water was added to prevent the interior of the bath from freezing) and all tubes were kept for 10 minutes at each degree from -1 to -16°C. 4 ml of pure water was put into each tube taken from the bath and these tubes were kept at 4°C for 24 hours. Later, the amount of ions transferred to pure water by thawing the frozen leaf in these tubes was measured with an electrical conductometer and the values were converted to cold damage according to the method of Griffith et al. (1992) and Taşgın et al. (2003).

Fresh weight (FW): Branch weights of the flowers used in the experiment were calculated daily by taking the difference between the weights of vases with and without flowers on a digital scale sensitive to 0.01 g. During the vase life of the flowers, proportional fresh

weight measurements were made at 2-day intervals (Day 1, Day 3, Day 5, Day 7, and Day 9). The following formula was used to calculate the proportional fresh weight (He et al., 2006).

FW (%) = (At/At=0) x 100

At: Branch weight on day t (e.g. 1st, 2nd, 3rd, etc.)

At=0: Initial (day 0) weight of the branch

Statistical Analysis

The study was set up with 3 replications according to the Randomized Plot Trial Design and a total of 189 flowers were used, 3 flowers in each replication. The numerical data obtained were subjected to analysis of variance (ANOVA) using the IBM SPSS version 25.0 package program, and the Duncan multiple comparison method ($p<0.05$) was used to determine the differences between the averages.

RESULTS and DISCUSSION

Vase life: In the ornamental plants sector, the vase life of cut flowers is one of the fundamental criteria for evaluating quality. The vase life of cut rose flowers is generally short due to both wilting of flowers and neck bending (Ünsal, 2022). In this research, the necessary cold transportation conditions were set up (+10 ºC) in the transportation of cut rose flowers from their production areas to the point where they will be marketed, and then the bacteria isolated from the leaf apoplast of the cold-tolerant plants (14 wild and 2 cultivated) were applied to the leaves of cut flowers of the rose plant (*Rosa hybrida* L. 'Samourai'). Among the bacterial applications, the lowest vase life was obtained from Control 1, B3, and K1 isolates. The applications with the longest vase life were determined numerically as D3, D4, M1, and K1 + B8. Both petals and green leaves maintained their (in D3, D4, M1, and K1 + B8 applications) vitality until the 9th day when the vase life period was evaluated. These applications, which have obtained the highest vase lifetimes, and B5, B8, D1, D2, K3, Ç1, Ç2, M2, and D1 + Ç1 applications were in the same statistical group (Table 3). When compared to Control 2, D3, D4, M1, and K1 + B8 applications extended the vase life of cut roses by 55.55%.

Table 3. Effects of the bacterial isolates on vase lifetime (days), visual quality scores, and % cold damage on leaves of cut roses

Çizelge 4. Bakteri izolatlarının kesme gül yapraklarında vazo ömrü (gün), görsel kalite puanları ve soğuk zarar yüzdesine etkileri.

Applications	Vase life (days)	Visual quality (1-5 scale)	Damage% (µS)
Control 1 (+2 ºC)	1.00±0.000 g ***	1.33	75.55±3.410 ns
Control 2 (+10 ºC)	5.00±1.000 ef	3.33	50.42±0.530
B1	6.00±1.000 cde	4.33	33.44±2.340
B3	1.00±0.000 g	4.33	29.83±1.880
B4	5.67±0.577 de	3.00	28.33±1.873
B5	8.00±1.732 ab	3.33	20.13±0.650
B6	4.00±1.000 f	3.33	49.25±0.820
B8	8.67±0.577 ab	2.67	23.26±0.280
D1	8.33±1.154 ab	4.67	40.47±0.480
D2	8.67±0.577 ab	5.00	12.63±1.150
D3	9.00±0.000 a	3.33	30.05±1.040
D4	9.00±0.000 a	4.67	32.20±0.934
K1	2.00±1.000 g	1.67	34.37±0.760
K2	7.00±1.000 bcd	3.00	33.62±0.730
K3	8.00±1.732 ab	4.00	24.69±0.730
G1	4.00±1.000 f	1.00	46.39±0.610
Ç1	8.00±1.000 ab	4.67	35.22±0.660
Ç2	7.33±0.577 abc	1.67	37.60±1.470
M1	9.00±0.000 a	3.67	25.07±0.270
M2	8.33±0.577 ab	2.67	99.50±0.866
K1 + B8	9.00±0.000 a	4.67	72.02±1.390
D1 + Ç1	7.67±1.527b	3.33	55.08±0.210
F value	F(25.112)= 0.000		F(741.975)= 0.000

ns: insignificant at $p>0.05$, statistically significant at probability level of ***$P<0.001$. Note: There is no difference between the means indicated with the same letter at the 5% significance level.

Some studies have reported that bacteria extend vase life, depending on the type of bacteria present in the vase solution (van Doorn et al., 1991; Jacob & Kim, 2010; Carlson et al., 2015). Carlson et al. (2015), reported that two bacterial strains (*Pseudomonas fulva* and *Escherichia coli*), also known as biocontrol bacteria, increased the vase life of cut *Zinnia elegans.* Additionally, Naing et al. (2017) reported that Enterobacter cloacae, a biocontrol bacterium, can extend the vase life of cloves by 3 days and plays an

important role in the biological control of microorganisms that cause petal senescence. Previous studies were generally carried out by adding beneficial bacteria to the vase solution. In this context, it was not possible to directly compare the present research results because we did not find the relevant literature.

Visual quality: Visual quality, the common day when deterioration started in all applications, was the 8th day of vase's life. Therefore, the visual quality parameter was evaluated on the 8th day of vase's life. In the present study, data regarding the visual quality scores of flowers are given in Table 3. It was determined that the flowers received 1 to 5 quality points on the scale created to evaluate the visual quality of the flowers. Among the applications, bacteria application D2 received the highest visual quality score (5 points). D1, D4, Ç1, K1, and K1 + B8 applications received 4.67 from this rating. In these applications, deformations began in both flowers and leaves. In general, cut roses maintained their visual quality criteria in the vase for 9 days with D2, D1, D4, Ç1, K1 and K1 + B8 applications. Control-2 and Ç2 applications dried out due to the climate chamber problem before they were taken into vase life trials, and the flowers belonging to the groups to which B5, B6, K1, and G1 bacterial isolates were applied started to bend before they fully opened, and neck bending continued in the flowers in the following days of vase life.

Some endophytic bacteria that live symbiotically with plants contain ACC deaminase (1-aminocyclopropane-1-carboxylic acid deaminase) enzyme, which prevents the synthesis of ethylene produced in the plant. Thanks to this enzyme, these bacteria suppress ethylene synthesis in the plant, causing positive effects on delaying senescence and longevity. ACC deminase activity of bacterial isolates isolated from cold-tolerant wild plants used in the research was determined as a result of the research conducted by Tiryaki (2015). It has been reported that there are differences in ACC deminase activity among isolates and that the bacterial isolate with the highest ACC deaminase activity is K2 [*Brevibacterium frigoritolerans* (DT-20)] (Tiryaki, 2015). As a result of the present research, the applications that received the highest visual quality scores, D2, D1, D4, Ç1, K1 and K1+B8, contain *Staphylococcus intermedius* (D1), *Bacillus cereus* (D2, D4), *P. fluorescens* (Ç1, K1) and *Bacillus cereus* + *S. kitahiroshimense* (K1 + B8) isolates.

Viability Test: Electrolyte leakage in tissues increases due to the damage to cell membranes after chilling or freezing as a result of low temperatures (Jha et al., 2019). Due to the increase in membrane permeability, the electrolyte exchange between cells and the external environment increases. Increased electrolyte leakage is directly related to chilling or cold damage (CD%) in cells and tissues (Campos et al., 2003). In this research, 18 different endophytic bacteria isolated from the leaf apoplast of cold-resistant wild plants and containing enzymes that inhibit ethylene synthesis in the plant were applied. When the CD% values on green leaves given in Table 3 are examined, the highest cold damage was determined in the application of Control 1 (+2 ºC). Leaf application of all bacterial isolates was made at +10 ºC. In this case, the cold damage determined to be 50% and above was determined in Control-2, M2, K1 + B8, and D1 + Ç1. The lowest CD% was determined in the D2 bacterial isolate (Table 3).

The cold damage parameter on leaves is generally used as a stress parameter. In the study, cold damage levels in leaves have been analyzed to determine stress tolerance or degree of protection from stress damage. In the literature, we have not come across a study that determines the DH% for a plant that is kept at a low temperature such as + 10 ºC for a certain period after PGPB is applied to a plant and then kept in a vase at room temperature conditions. Therefore, it is not possible to directly compare the data with a similar study. Studies are determining the effects of PGPBs applied under low-temperature conditions on plant %DH (Ait Barka et al., 2006; Turan et al., 2013; Tiryaki, 2015). However, the present study's subject and purpose are different from these studies and comparisons cannot be made. Psychrophile (cold-loving) bacteria, which can live at low temperatures and have high adaptations to tolerate this, have reproductive temperatures between 0-4°C, and their enzymes can show activity between –5°C and +20°C (Arda, 2000). Many researchers have isolated psychrophile microorganisms from various environments such as seas, oceans, soils, fish, milk, meat, and vegetables (Graumann, 1996). It was concluded that the psychrophile (cold-loving) bacteria used in the present study, especially D2, has been reached and can be applied in the transportation of cut flowers.

It has been shown that psychrophile PGPBs isolated from cold-tolerant wild plants can increase low-temperature tolerance in some plants (Selvakumar et al., 2008). In addition to surviving at extremely low temperatures, "psychrophiles" also secrete active biomolecules that can promote plant growth in low-temperature ranges (Rondón et al., 2019; Yadav et al., 2016; Balcázar et al., 2015). In the D2 application, where the minimum CD% was determined, the best visual quality score was also determined. It is thought that this practice may have played a role in increasing tolerance to water loss in cut roses.

Fresh weight (FW): The effect of different bacterial isolate applications on relative fresh weight and the weight changes that occurred during the 9-day vase period are given in Figure 3.

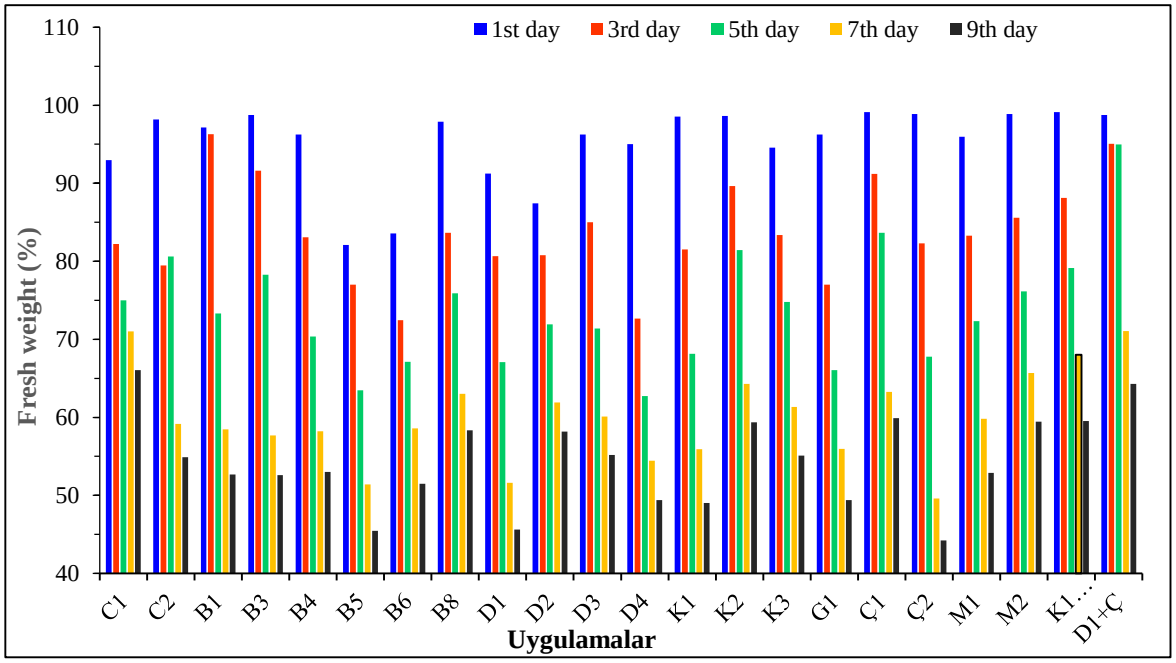

Figure 3. Effects of the bacterial isolates on the relative fresh weight (%) of cut roses. C1: Control (+2 °C); C2: Control (+10 °C). (*F values:* 1st day F(2.023)=0,24; 3rd day F(1.290)=0,233; 5th day F(2.159)=0,016; 7th day F(1.101)=0,382; 9th day F(1.225)=0,278). Statistically significant at probability level of P<0.05.

Şekil 3. Bakteri izolatlarının kesme güllerin bağıl taze ağırlığı (%) üzerine etkileri.

It was determined that the applications were significantly effective (p≤0.001) on the relative FW change of cut rose. At the end of the first day of vase life, the highest relative FW increase among the applications was determined in all applications except the application groups in which B5 and B6 bacterial isolates were applied. The lowest relative FW increase was seen in the B5 application. At the end of the 9th day of the study, the highest FW weight increase was detected in Control 1 (66.06%) and D1+Ç1 applications (64.28%) (Figure 3).

Researchers reported that the proportional fresh weight increases in cut roses were first between days 3-9 st and started to decrease after the 9th day (Ichimura et al., 1999; Alaey et al., 2011; Tuna, 2012). Ichimura et al. (2002) also reported that the relative FW of cut roses increased until the 3rd day in control and until the 6th day in different flower preservatives. Although the results obtained in the present study are generally compatible with the above literature, it is observed that the proportional FW change varies according to bacterial isolates. It is thought that this may be due to the variety used and trial conditions. In the current study, only tap water was placed in the vases of all application groups as a vase solution. The flower stems of the cut roses at the beginning of the experiment were cut to a length of 1-1.5 cm and included in the experiment. The decrease in fresh weight ratios has nothing to do with nutritional or vase solution content. We think that the proportional changes in FW may be due to +10 ºC conditions not being optimum. Especially Control-2 and Ç2 applications suffered from drying damage to petals and leaves before they were placed in the vase. Additionally, we think that the shedding of green leaves, which could not be foreseen before the experiment, may be misleading in the measurement of these weights. The present research, in which a method that has not been done or applied before, is the first of these important studies. We believe that it can serve as a guide for future studies.

CONCLUSION

In the current research, bacteria isolated from the leaf apoplast of 16 different cold-resistant plants were separately applied to the plant leaves to partially create the necessary cold transportation conditions for the transportation of cut rose flowers from the greenhouse to the point where they will be marketed. At the same time, two different bacterial formulations prepared with two binary bacterial strains were applied. The effects of the applications on the parameters affecting the vase life of cut roses were examined and evaluations were made. It has been determined that applications in which D3, D4, M1, and K1 + B8 bacterial isolates are used in the transportation of cut flowers are promising applications in terms of ensuring less cooling, minimal loss in cut flower quality, extending vase life, and reducing cooling costs. In general, with D2, D1, D4, Ç1, K1, and K1+B8 applications, cut roses maintained their visual quality criteria in the vase for 9 days. The present study is the first to aim to provide less cooling and extend vase life by using psychrophile PGPBs in

the transportation of cut flowers. Research should be continued by enriching the number and content of studies for this purpose.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Ataturk University Scientific Research Unity, Turkey for funding this research work through project number FHD-2022-10888.

Author contribution statement

DT is the primary author of this article. Investigation, Methodology DT, FPK; Formal analysis, and Writing-Original Draft – FPK, ÖA, DT; References, Data Collection and/or Processing – DT; Analysis and/or Interpretation, Critical Review and Edit- AD. All authors read and approved the final manuscript.

Declaration of Interests

The authors declare that there are no potential conflicts of interest regarding the authorship, research, and publication of this manuscript.

KAYNAKLAR

Ait Barka, E., Nowak, J. & Clément, C. (2006). Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. *Applied and environmental microbiology, 72*(11), 7246-7252.

Alaey, M., Babalar, M., Naderi, R. & Kafi, M., (2011). Effect of pre-and postharvest salicylic acid treatment on physio-chemical attributes in relation to vase-life of rose cut flowers. *Postharvest Biology and Technology, 61*, 91–94.

Anonymous (2022). SAMOURAÏ® Meikatana, Characteristics. https://meilland.com/en/catalogue/samourai-meikatana/ (Acces date: 07.08.2022)

Arda, M. (2000). Temel Mikrobiyoloji. Medisan Yayın Serisi no:46, ANKARA.

Atici, Ö. & Nalbantoglu, B. (1999). Effect of apoplastic proteins on freezing tolerance in leaves. *Phytochemistry*, 50, 755-761.

Balcázar, W., Rondón, J., Rengifo, M., Ball, M.M., Melfo, A., Gómez, W. & Yarzábal, L.A. (2015). Bioprospecting glacial ice for plant growth promoting bacteria. *Microbiol. Res. 177*, 1–7.

Barlas, S., Kart, M. Ç. Ö., Kınıklı, F. & Işın, S. (2019). Factors affecting the production decisions of cut flowers producers in Menderes region of Izmir province. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi/Atatürk University Journal of Agricultural Faculty, 50*(3), 231-238.

Campos, P., Quartin, V., Ramalho, J.C. & Nunes, M.A., (2003). Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of Coffea sp. plants. *J. Plant Physiolol., 160*, 283-292.

Carlson, A. S., Dole, J. M., Matthysse, A. G., Hoffmann, W. A. & Kornegay, J. L. (2015). Bacteria species and solution pH effect postharvest quality of cut *Zinnia elegans*. *Sci. Hortic. 194*, 71–78. doi: 10.1016/j.scienta.2015.07.044

Çelikel, F. G. (2020). Postharvest Quality and Technology of Cut Flowers and Ornamental Plants. *Black Sea Journal of Agriculture, 3*(3), 225-232.

Da Silva, J. T. (2003). The cut flower: postharvest considerations. *J. Biol. Sci, 3*(4), 406-442.

Graumann, P., Schroder, K., Schmid, K. & Marahiel, M.A. (1996). Cold Shock stres induced proteins in *Bacillus subtilis*. *J. Bacteriol. 178*, 4611-4619.

Griffith, M., Ala, P., Yang, D. S., Hon, W. C. & Moffatt, B. A. (1992). Antifreeze protein produced endogenously in winter rye leaves. *Plant Physiology*, *100*(2), 593-596.

He, S., Joyce, D. C., Irving, D. E. & Faragher, J. D. (2006). Stem end blockage in cut Grevillea 'Crimson Yul-lo'inflorescences. *Postharvest Biology and Technology, 41*(1), 78-84.

Ichimura, K., Kawabata, Y., Kishimoto, M., Goto R. & Yamada, K., (2002). Variation with the cultivar in the vase life of cut rose flowers. *Bulletin of the National Institute of Floricultural Science, 2*, 9-20.

Ichimura, K., Kojima, K. & Goto, R., (1999). Effects of Temperature, 8-hydroxyquinoline sulphate and sucrose on the vase life of cut rose flowers. *Postharvest Biology and Technology, 15*: 33–40.

Jacob, B. M. & Kim, E. (2010). Inhibiting biofilm formation of *Enterobacter* sp. prevented premature withering in cut flowers. *Korean J. Chem. Eng. 27*, 1252–1257. doi: 10.1007/s11814-010-0196-5

Jahnke, N. J., Dole, J. M., Bergmann, B. A., Ma, G. & Perkins-Veazie, P. (2020). Extending cut *Paeonia lactiflora* pall. Storage duration using sub-zero storage temperatures. *Agronomy, 10*(11), 1694.

Jahnke, N. J., Kalinowski, J. & Dole, J. M. (2022). Postharvest Handling Techniques for Long-term Storage of Cut Tulip and Dutch Iris. *Horttechnology, 32*(3), 263-274.

Jha, P. K., Xanthakis, E., Chevallier, S., Jury, V. & Le-Bail, A. (2019). Assessment of freeze damage in fruits and vegetables. *Food Research International, 121*, 479-496.

Kazaz, S. (2015). Kesme Çiçeklerde Hasat Sonrası Ömrü Etkileyen Faktörler. *Türkiye Tohumcular Birliği Dergisi, 4* (14): 42-45, Nisan-Haziran 2015.

Lü, P., He, S., Li, H., Cao, J. & Xu, H. L. (2010). Effects of nano-silver treatment on vase life of cut rose cv. Movie Star flowers. *J. Food Agric. Environ, 8*(2), 1118-1122.

Naing, A. H., Win, N. M., Han, J. S., Lim, K. B. & Kim, C. K. (2017). Role of nano-silver and the bacterial strain *Enterobacter cloacae* in increasing vase life of cut carnation 'Omea'. *Frontiers in Plant Science, 8*, 1590.

Onozaki, T., Ikeda, H. & Yamaguchi, T. (2001). Genetic improvement of vase life of carnation flowers by crossing and selection. *Scientia Horticulturae, 87*(1-2), 107-120.

Rondón, J.J., Ball, M.M., Castro, L.T. & Yarzábal, L.A. (2019). Eurypsychrophilic *Pseudomonas* spp. Isolated from Venezuelan tropical glaciers as promoters of wheat growth and biocontrol agents of plant pathogens at low-temperatures. *Environ. Sustain., 2*, 265–275.

Selvakumar, G., Kundu, S., Piyush, J., Sehar, N., Gupta, A.D., Mishra, P.K. & Gupta, H.S., (2008). Characterization of a cold tolerant plant growth promoting bacterium *Pantoea dispersa* 1A, isolated from a subalpine soil in the North WesternIndian Himalaya. *World J Microbiol Biotechnol 24*,955–960.

Sönmez, F. (2012). Cut Flower Sector Report. Eastern Black Sea Development Agency. (Kesme Çiçek Sektör Raporu. Doğu Karadeniz Kalkınma Ajansı).

Taşgın, E., Atící, Ö. & Nalbantoğlu, B. (2003). Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves. *Plant Growth Regulation, 41*, 231-236.

Tiryaki, D. (2015). The effects of bacterıa ısolated from the leaf apoplast of cold-resıstant plants on the cold stress tolerance of bean (*Phaseolus vulgaris* L.). Atatürk University Institute of Science Department of Biology Department of Botany, Ph.D. Thesis, 167 p.

Tiryaki, D., Aydın, İ. & Atıcı, Ö. (2019). Psychrotolerant bacteria isolated from the leaf apoplast of cold-adapted wild plants improve the cold resistance of bean (*Phaseolus vulgaris* L.) under low temperature. *Cryobiology, 86*, 111-119.

Tuna, S. (2012). Kesme gül ve gerbera çiçeklerinin vazo ömrünü artırmak için bazı uçucu yağlar ve ana bileşenlerinin kullanım olanakları. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü, Isparta.

Turan, M., Güllüce, M., Çakmak, R. & Şahin, F. (2013). Effect of plant growth-promoting rhizobacteria strain on freezing injury and antioxidant enzyme activity of wheat and barley. *Journal of Plant Nutrition, 36*(5), 731-748.

Ueyama, S. & Ichimura, K. (1998). Effects of 2-hydroxy-3-ionene chloride polymer on the vase life of cut rose flowers. *Postharvest Biology and Technology, 14*(1), 65-70.

Ünsal, H.T. (2022). Effect of postharvest pre-treatments on flower quality and vase life of cut rose (*Rosa hybrida* L.) flowers. Ankara University, Graduate School of Natural and Applied Sciences, Department of Horticulture, M.Sc. Thesis, 59 p.

van Doorn, W. G., Zagory, D., de Witte, Y. & Harkema, H. (1991). Effects of vase-water bacteria on the senescence of cut carnation flowers. *Postharvest Biol. Technol. 1*, 161–168. doi: 10.1016/0925-5214(91)90008-Y

Yadav, A.N., Sachan, S.G., Verma, P., Kaushik, R. & Saxena, A.K. (2016). Cold active hydrolytic enzymes production by psychrotrophic bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. *J. Basic Microbiol., 56*, 294–307.