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Micropropagation of Gerbera (*Gerbera jamesonii* Bolus) under Different Color of Light-Emitting Diodes

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In vitro rooting,
Acclimatization

Abstract: In present research, the effects of light quality on micropropagation of gerbera were investigated. The MS medium containing 1 mg L^{-1} BAP and 0.1 mg L^{-1} NAA was used in proliferation stage and the MS medium containing 0.05 mg L^{-1} IBA was used in rooting stage of the study. Cultured plants were grown in cabinets illuminated with red, blue, daylight LED lamps separately and their various mixtures. Cool white fluorescent lamp was used as control treatment in the study. The highest ratio of explant with new shoots, the number of shoots per explant and shoot length values were obtained from cool white fluorescent lamps (90%, 5.35 shoots and 2.63 cm, respectivey), 100% blue LED lamp (95%, 5.24 shoots and 2.40 cm, respectively), 100% cool white LED lamp (90%, 4.18 shoots and 2.21 cm, respectively) and 70% red + 30% blue LED lamp treatments (80%, 4.14 shoots and 2.21cm, respectively) in the proliferation stage of the study. These treatments also yielded the highest values in terms of plant fresh and dry weight. While the highest rooting percentage (75%) was obtained from 100% cool white LED light treatment, the differences between the treatments except for 100% red LED light were not significant. Rooted plants were successfully acclimatized to outdoor conditions with 84% survival rate in peat + perlite mixture (2:1).

Gerberanın (*Gerbera jamesonii* Bolus) Farklı Renklerde Işık Yayan LED Lambalar ile Aydınlatılmış Koşullarda Mikroçoğaltımı

Anahtar Kelimeler
Gerbera,
Gerbera jamesonii Bolus,
Mikroçoğaltım,
Işık kalitesi,
In vitro köklenme,
Dış koşullara alıştırma

Özet: Bu çalışmada gerberanın mikroçoğaltımı üzerine ışık kalitesinin etkileri araştırılmıştır. Araştırmanın çoğaltma aşamasında 1 mg L^{-1} BAP ve 0.1 mg L^{-1} NAA, köklendirme aşamasında ise 0.05 mg L^{-1} IBA içeren MS ortamı kullanılmıştır. Kültüre alınan bitkiler; kırmızı, mavi ve gün ışığı yayan LED lambaların yalın ve değişik oranlarda karışımları ile aydınlatılmış kabinlerde geliştirilmiştir. Beyaz fluoresan lamba kontrol olarak kullanılmıştır. Çoğaltma aşamasında; en yüksek yeni sürgün oluşturan eksplant oranı, sürgün sayısı ve sürgün uzunluğu değerleri beyaz fluoresan lamba (sırasıyla, %90, 5.35 adet ve 2.63 cm), %100 mavi LED lamba (sırasıyla, %95, 5.24 adet ve 2.40 cm), %100 soğuk beyaz LED lamba (sırasıyla, %90, 4.18 adet ve 2.21 cm) ve %70 kırmızı + %30 mavi LED lamba (sırasıyla, %80, 4.14 adet ve 2.21 cm) uygulamalarından elde edilmiştir. Bu uygulamalar, bitki yaş ve kuru ağırlıkları bakımından da en yüksek değerleri vermiştir. Çalışmada, %100 kırmızı LED lamba uygulamasının köklenmeyi azalttığı belirlenmiştir. Köklenmiş bitkiler torf+perlit (2:1) karışımı içeren saksılara şaşırtılarak %84 oranında dış koşullara alıştırılmıştır.

1. Introduction

Gerbera, which has a significant share in cut flower production of Turkey, is not propagated with seeds due to its heterozygous structure [1]. Therefore, vegetative propagation methods must be used in proliferation of gerbera. Gerbera is generally propagated by the method of separation of rhizomes. However, this method has some problems, because it is quite slow and does not provide virus-free seedlings. Therefore, tissue culture methods have been used as an alternative vegetative propagation

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technique. With these methods, identical, strong and disease-free plants can be reproduced any time of the year [2]. The most important problem in micropropagation of gerbera is infections throughout the initial phase of the method [3]. Furthermore, the formation of flower buds on shoot apex is also an important problem when the shoot tips are used as explants. Recently, some studies on micro-propagation of gerbera have been successfully carried out [4-7]. However, alternative studies are being carried out in order to make more economical micro-propagation in gerbera. Chemicals and energy are the two major cost items in micropropagation. Electricity costs constitute a major portion (approximately 25-30%) of the total cost in micropropagation studies. In recent years, LED lamps consuming less energy has been started to be used for the illumination of climate chambers. With the use of LED lamps, the illumination possibility with a desired light color of the plants is also provided. There are few studies about the use of LED lamps producing light of different colors. Wang et al. [8] reported that red light increased the shoot length more than blue and cool white light in gerbera. It is also understood from some studies that red light increased the internode length of shoots [9], accelerated the flowering in plants [10] and has a positive effect on seed germination [11-13]. It was stated that the red light used together with the blue light was more effective on plant development and photosynthesis although red light used alone was not effective [14, 15]. It was not encountered a detailed study about in vitro using of LED lamps produced day light.

This study was carried out to determine the effects of different light colors on micropropagation of gerbera cultivar 'Rosalin'. For this purpose, the effects of blue, red, day light colors and their various mixtures on in vitro proliferation and rooting of gerbera were investigated.

2. Material and Method

2.1. Materials

2.1.1. Plant material

The in vitro plants of gerbera cultivar 'Rosalin' with pink flowers belonging to *Gerbera jamesonii* Bolus species were used as the plant material of the present study.

2.1.2. Cabinets

Cabinets designed in the size of 50x50x30 cm were used for incubation of the plants under different light colors. The bottom and side surfaces of the cabinets are made of chipboard and the upper surface is made of aluminum. Over side surfaces, 15 holes in diameter of 1.5 cm were opened and a fan with 4 cm diameter was connected to one side of cabinets in order to ensure the sufficient ventilation. LED lamps were located on the ceiling of the cabinets [16]. Wavelengths of lights produced by LEDs used in the study were measured as between 400-700 nm for cool white and daylight, 620-630 nm for red light and 455-475 nm for blue light. Cool white fluorescent lamps were used as control treatments of the study.

2.2. Methods

Murashige and Skoog (MS) basal medium [17] was used in both proliferation and rooting stages of the study. MS medium was supplemented with 1 mg L^{-1} BAP and 0.1 mg L^{-1} IBA at the proliferation stage and supplemented with 0.05 mg L^{-1} IBA at the rooting stage. The pH of the medium was adjusted to 5.7 and then 30 g L^{-1} sucrose and 7 g L^{-1} agar were added into the medium. The medium was distributed into Erlenmeyer flasks (size in 250 ml) as 50 ml in each one and autoclaved at 121 ºC for 15 min.

2.2.1. The effects of light quality on proliferation

At this stage, the plants were cultured in cabinets illuminated with red, blue, daylight LED lamp separately and their various mixtures (Table 1). Inside temperature of the cabinets was recorded in every 3 min via HOBO U12-012 data loggers during the incubation period and average temperatures were calculated. In addition, DELTA OHM 9847K data recorder was used for the measurement of the light intensity in the cabinets and the light intensity of inside was set to 140±10 mmol / m^2s. At the end of 4 weeks incubation period, the alive explant percentage (%), the percentage of explants with new shoots (%), the number of shoots per explants (shoots), shoot length (cm), plant fresh (g) and dry weight (g) values were determined. Plant fresh and dry weights were determined using precision scales (±0.0001, Sartorious). Before the fresh weight of the plants was determined, the plants were washed in tap water in order to remove the residues of nutrient medium and the moisture on plants were removed with filter paper. After the plants were kept in an oven set at 65°C temperature for 3 days, the dry weight was determined.

2.2.2. The effects of light quality on in vitro rooting

In order to determine the effect of light quality on in vitro rooting, the plants were cultured in cabinets illuminated with red, blue, daylight LED lamp separately and their various mixtures for 6 weeks. The rooting percentage, number of roots per explant and the root length were determined at the end of 6 weeks.

2.2.3. Acclimatization of rooted plants

Rooted plantlets were washed in tap water and then held in 5% fungicide solution (Bestnate, 500 g L^{-1}

Imazalil) for 2 min. The plantlets were planted in plastic bottles containing peat and perlite mixture (2:1,w/w) in order to be acclimatized to outer conditions. The bottles were covered to maintain high humidity. And then, the same rate fungicide solution was sprayed to the leaves and the bottom part of the plants 5 times in 4 days intervals in order to prevent future infections. The bottles were opened after 3 weeks and then the percentage of survived plants was recorded for 6 weeks after potting.

2.3. Experimental design and data analysis

Experiments were conducted in completely randomized design with 4 replications with 5 plants in each. The data were subjected to analysis of variance (ANOVA) using MINITAB software package and the means were separated by Tukey test ($p<0.05$). An arcsine transformation was performed to stabilize the variance of percent data, and angle values were used in the analysis of variance.

3. Results and Discussion

3.1. The effects of light quality on proliferation

The effects of different light colors on alive explant ratio, the ratio of explants with new shoots, number of shoots and shoot length of gerbera cultivar 'Rosalin' are provided in Table 1. The treatments of 33% RL + 33% BL + 33% DL and 50% RL + 50% BL yielded lower alive explant ratios than other treatments. Although it was not significant, the treatments of 100% RL (100%) and 100% BL (95%) yielded higher values than control treatment (CWFL) (90%). However, since the rate of explants with new shoots and number of shoots are more important than the alive explant rate, such an effect of red light was not found to be significant. Despite the highest alive explant rate in 100% RL treatment, it was observed that plants were quite unhealthy and turned to yellow. The reason of the highest survival rate in red light application might be resulted from faster plant growth because of its prolonging effect on internodes [9]. In present study, 70% RL+30% BL, 60% RL+ 40% BL and 40% RL+30% BL+30% DL treatments yielded similar results with the control treatment. Lian et al. [18] investigated the effects of white, red, blue, red+blue light treatments and dark conditions on offspring onion formation in lily and reported the highest alive explant rate for white light treatments.

The highest ratio of explants with new shoots was obtained from 100% BL (95%), CWFL (90%) and 100% WL (90%) treatments. The value was interestingly the lowest (35%) in the red light treatment, although the alive explant rate was the highest (100%) under red light. Very few axillary shoot formations in red light treatments indicated that the red light had stimulating effects on apical bud dominance in gerbera. However, it was reported that the level of auxin was lower and the level of gibberellin was higher under red light than under blue light [19]. Decreasing chlorophyll levels were also reported for plants grown under red light [14].

The highest number of shoots per explant (5.35 shoots) was obtained from the control treatment (CWFL). However, the differences between the treatments of 100% BL, 50% RL+50% BL and 100% WL (5.24, 4.44 and 4.18 shoots, respectively) and the control treatment were not significant. Such finding revealed that current treatments could be an alternative to the control treatment. It was reported that LED lamps may provide 1.5-4 times savings in electricity depending on the color of light compared to use of fluorescent lamps [16]. Since the electricity is a major cost item in micropropagation, significant savings can be achieved through LED lamps. Similar to current findings, Lian et al. [18] reported increased new offspring onion formation with blue, white and red+blue colors. In addition, Kim et al. [9] stated that applications of red+blue LED lamps and white fluorescent lamps increased the activity of photosynthesis compared to other light colors in chrysanthemum. High number of shoots under different colors might be related to the increase in photosynthesis rates. Poudel et al. [20] used blue and red LED lamps in in vitro proliferation of grapevine and reported similar results with the present study in terms of number of shoots compared to cool white fluorescent lamps. Besides the color of light, it was reported that the light intensity and the period of illumination might be effective on micropropagation of plants [14]. Unlike the current finding, number of shoots was the highest in plants illuminated with red light compared to other light colors [21] which might be partially due to genotype, light intensity and composition of medium. The success in in vitro proliferation of gerbera varied based on the genotypes [4]. Therefore, it could be stated that light quality and the use of growth regulators could exhibit different effects on different genotypes [22].

The highest shoot length (2.83 cm) was found in 100% RL treatment. This treatment was followed by CWFL and 100% BL treatments and the differences between the treatments were not significant. It was expressed in many studies that red light increased the lengths of internode, leaf and petiole in plants. Similar to current findings, Nhut et al. [14] reported that the longest plants in strawberries were obtained from the red-light treatments. The higher shoot length in red light treatments compared to other light colors may be related to endogen hormone levels. Indeed, Ouyang et al. [19] stated that the plants grown under red light contained more gibberellin than those grown under other light colors. It is known that gibberellins increase the length of internode in plants.

Table 1. The effects of light quality on alive explant ratio, the ratio of explants with new shoots, number of shoots and shoot length in gerbera cultivar 'Rosalin'

Treatments	Average temperature in cabinets (°C)	Ratio of alive explants (%)	Ratio of explants with new shoots (%)	Number of shoots	Shoot length (cm)
Cool white fluorescent lamp (CWFL)	27.8±2.38	90 ab*	90 ab	5.35 a	2.63 ab
LED Lamps					
100% Day Light (DL)	28.8±2.13	80 ab	55 bc	1.85 cd	2.10 bcd
100% Red Light (RL)	26.1±1.22	100 a	35 c	1.50 d	2.83 a
100% Blue light (BL)	27.0±1.50	95 ab	95 a	5.24 a	2.40 abc
100% White light (WL)	27.6±1.70	90 ab	90 ab	4.18 abc	2.21 bcd
33% RL + 33% BL + 33% DL	27.2±1.57	50 c	40 c	2.00 cd	1.73 d
40% RL + 30% BL + 30% DL	27.0±1.81	90 ab	75 abc	3.17 abcd	2.11 bcd
50% RL + 25% BL + 25% DL	27.6±1.51	80 ab	65 abc	3.81 abcd	1.96 cd
50% RL + 50% BL	26.6±1.24	70 bc	60 bc	4.44 ab	1.91 cd
60% RL + 20% BL + 20% DL	26.9±1.64	80 ab	45 c	1.97 cd	2.12 bcd
60% RL + 40% BL	28.0±1.78	85 ab	55 bc	2.20 bcd	1.84 cd
70% RL+ 15% BL + 15% DL	27.5±1.79	80 ab	70 abc	2.32 bcd	2.26 bcd
70% RL + 30% BL	27.4±1.60	90 ab	80 abc	4.14 abc	2.21 bcd

*The means indicated with different letters in the same column are significantly different by Tukey test (P<0.05)

The treatments of 100% RL, 33% RL+ 33% BL+33% DL and 100%DL yielded the lowest values in terms of plant fresh and dry weight (Table 2). Although the differences between other applications were not significant, the highest plant fresh weight values were obtained from 100% BL (6.82g), 100% WL (6.22g), 70% RL+30% BL (6.19g) and CWFL (5.82g) treatments. Similar to current findings, Kim et al. [9] in a study about micropropagation of chrysanthemum reported the highest plant fresh and dry weights for red + blue LEDs and white fluorescent lamp. Likewise, it was reported that the most effective treatments were white fluorescent lamp and 70% RL+30% BL with regard to fresh weight in a study on micropropagation of gerbera [8]. It is also expressed by Tanaka et al. [23] for Cymbidium plants and by Lian et al. [18] for lilium plants that white fluorescent lamp and red+blue LED treatments increased the fresh and dry weights. It is thought that red + blue lights increased the fresh and dry weight of plants because of their increasing effect on photosynthesis activity. Indeed, Shin [24] reported that the application of red + blue light increased the contents of chlorophyll, carotenoids and carbohydrates (glucose, fructose, sucrose and starch) more than other light applications in Doritaenopsis spp. It was also reported that the chlorophyll content in strawberry plants was higher in 70% RL+30% BL treatment than those of other applications [14].

3.2. The effects of light quality on in vitro rooting

When the effects of different light colors on in vitro rooting of gerbera were assessed, it was observed that the highest rooting percentage was obtained from 100% WL (75%) treatment (Table 3). However, the differences in rooting ratios of the other treatments except for 100% RL were not significant.

Table 2. The effects of light quality on fresh and dry weights in gerbera cultivar 'Rosalin'

Treatments	Plant fresh weight (g)	Plant dry weight (g)
Cool white fluorescent lamp(CWFL)	5.82 ab*	0.53 abc
LED Lamps		
100% Day Light (DL)	4.56 bc	0.43 c
100% Red Light (RL)	3.21 c	0.32 d
100% Blue light (BL)	6.82 a	0.63 a
100% White light (WL)	6.22 ab	0.57 ab
33% RL + 33% BL + 33% DL	4.16 bc	0.46 bc
40% RL + 30% BL + 30% DL	5.70 ab	0.54 abc
50% RL + 25% BL + 25% DL	5.11 abc	0.52 abc
50% RL + 50% BL	5.40 ab	0.56 ab
60% RL + 20% BL + 20% DL	4.86 abc	0.53 abc
60% RL + 40% BL	5.30 ab	0.54 abc
70% RL+ 15% BL + 15% DL	5.11 abc	0.53 abc
70% RL + 30% BL	6.19 ab	0.59 a

*The means indicated with different letters in the same column are significantly different by Tukey test (P<0.05)

It was determined that 100% RL treatments had a negative effect on in vitro rooting of gerbera. Poudel et al. [20] investigated the effects of white fluorescent lamp, blue LEDs and red LEDs on rooting of 3 grapevine rootstocks. Researchers reported that applications of blue and red LEDs increased the rooting compared to application of white fluorescent lamp in two of the rootstocks and they were not effective on the rooting of other rootstock. This suggests that the effects of the light quality on rooting varied based on genotypes. Iacona [25] stated that the light color was not effective on rooting of cherry rootstock 'Colt' and a successful rooting was dominantly depending on exogenous hormone

applications. It was also reported by Wang et al. [8] that the light color was not effective on in vitro rooting of gerbera. Unlike the current findings, Gabryszewska [21] reported that the most effective light on in vitro rooting of gerbera was red light. Researchers stated that red and green lights were more effective than blue, white and white +UV lights on rooting of gerbera, but in order to see that effect, the addition of IAA into the growth medium was absolutely necessary.

Different colors of light were not found to be significantly effective on number of roots per explant of the present study. The highest number of roots (3.48) was obtained from 100% WL treatment, however the differences between the treatments except for 100% RL were not significant (Table 3). The decrease in root numbers in 100% RL treatment may be resulted from reduced chlorophyll content of leaves [14]. Iacona [25] investigated the effects of red, blue, white and red + blue LEDs on in vitro rooting of cherry rootstock 'Colt' and reported that the highest number of roots was obtained from blue+red LEDs. Similarly, the blue and red+blue lights significantly increased the number of roots compared to the red-light treatments of the present study. It was understood from previous researches that the effects of light quality on rooting varied considerably with the species and cultivars.

Table 3. The effects of light quality on rooting percentage, number of roots and root length in gerbera cultivar 'Rosalin'.

Treatments	Average temperature in cabinets (°C)	Rooting percentage (%)	Number of roots	Root lenght (cm)
Cool white fluorescent lamp (CWFL)	26.7±2.24	70.0 a*	2.88 a	8.10 a
LED Lamps				
100% Day Light (DL)	26.9±1.87	55.0 ab	2.12 ab	6.24 abc
100% Red Light (RL)	24.8±1.18	20.0 b	0.63 b	4.09 bc
100% Blue light (BL)	25.8±1.65	60.0 ab	3.02 a	4.90 abc
100% White light (WL)	26.2±1.79	75.0 a	3.48 a	5.53 abc
33% RL + 33% BL + 33% DL	26.1±1.44	50.0 ab	2.54 a	5.79 abc
40% RL + 30% BL + 30% DL	26.1±1.68	55.0 ab	1.78 ab	6.64 abc
50% RL + 25% BL + 25% DL	26.4±1.65	46.7 ab	3.00 a	5.55 abc
50% RL + 50% BL	25.4±1.18	53.3 ab	2.89 a	6.18 abc
60% RL + 20% BL + 20% DL	25.8±1.50	55.0 ab	2.79 a	5.69 abc
60% RL + 40% BL	27.0±2.06	60.0 ab	2.40 ab	5.48 abc
70% RL+ 15% BL + 15% DL	26.4±1.84	35.0 ab	3.25 a	7.46 ab
70% RL + 30% BL	26.9±1.92	46.7 ab	1.78 ab	3.96 c

*The means indicated with different letters in the same column are significantly different by Tukey test (P<0.05)

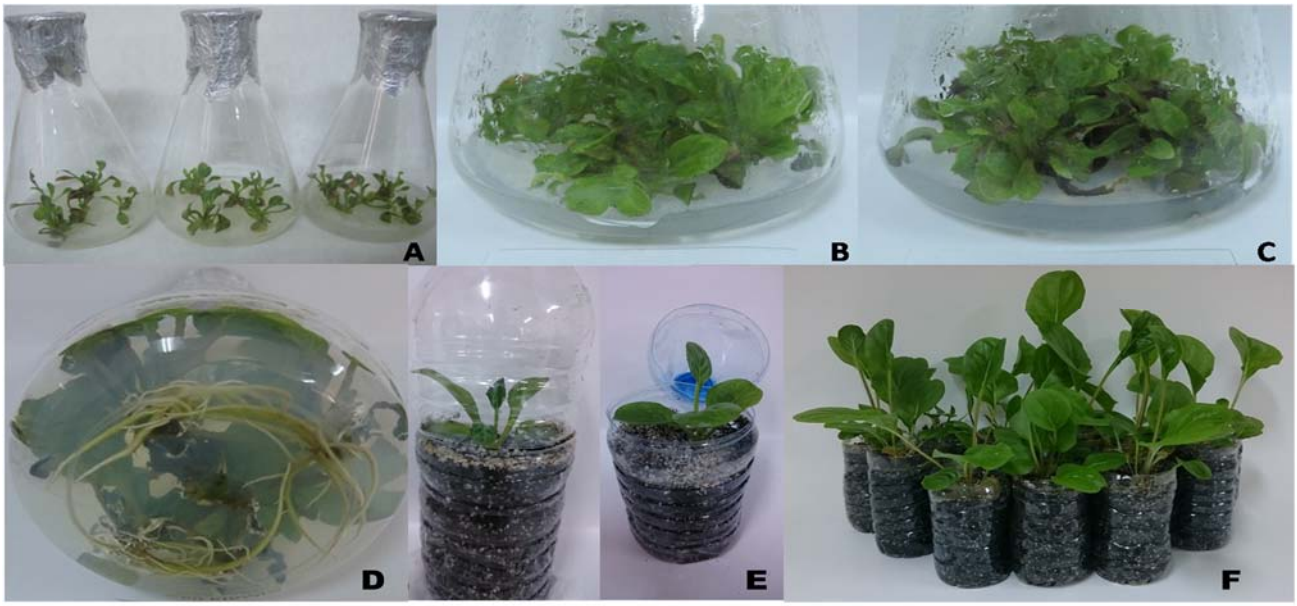

Figure 1. Micropropagation stages of gerbera cultivar 'Rosalin'. A; Cultured plants on MS medium containing 1 mg L^{-1} BAP and 0.1 mg L^{-1} IBA, B; Proliferated plants in cabinet illuminated with cool white fluorescent lamp, C; Proliferated plants in cabinet illuminated with 100% blue LED lamps, D; Rooted plants in cabinet illuminated with 100% blue LED lamps, E; Acclimatization stages of the plants (1 and 21 days after planting, respectively), F; Acclimatized plants (42 days after planting in pots).

Significant differences were not observed in root lengths of the treatments except for 100% RL and 70% RL+30% BL treatments. The highest root length (8.10 cm) was obtained from CWFL treatment (Table 3). Similarly, Gabryszewska [21] reported that different light colors were not effective on root lengths when the nutrient medium without plant growth regulators was used. Researchers stated that the lowest root length was obtained from red light treatment when IAA was added to the nutrient medium. It was also reported in studies by Wang et al. [8] and Poudel et al. [20] that light colors were not effective on root length. However, the effects of light color on root length could vary based on various factors such as light intensity, genotype and the composition of growth medium (with/without plant growth regulators).

3.3. Acclimatization of rooted plants

The rooted plants were successfully acclimatized to outer conditions with a survival rate of 84%. Generally, there were not much problems about the acclimatization of gerbera. Indeed, it was reported in previous studies that the success rate was mostly over 90% in gerbera [4, 26, 27].

4. Conclusion

As a result, it was concluded that 100% BL, 100% WL and 70% RL+30% BL treatments could be used as an alternative to widely used white fluorescent lamps for micropropagation of gerbera cultivar 'Rosalin'. The most important advantage of these treatments is their more economical nature than cool white fluorescent lamps in term of electricity cost.

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