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Response of Two *Rosa sp.* to Light Quality *in Vitro*.

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Abstract :

In this study, dark and various light qualities (white, red, green, and blue) were applied to evaluate their effects on growth characteristics, chemical content, and callus characteristics of *Rosa damascene* Mill. and *Rosa hybirda* L.

Explant (single-node and shoot tips) cultured on MS media supplemented with sucrose, agar, and plant growth regulators (Kin 0.5 mg/l and IBA 1 mg/l for whole plant formation experiment or 1 mg/l kin with 0.5 mg/l IBA for callus experiment), incubated in a growth chamber .

The results of the whole plant formation experiment showed variation in growth characteristics in two types of Rosa, Green and white light caused the height ratio of shoot growth compared with dark, while red and green light caused the height ratio of root growth to compare with dark. Rosa growing in white or green has a higher content of chlorophyll, as well as carbohydrates and proteins in shoot and root parts compared with dark.

The results of the callus experiment explained variation in callus characteristics (shape, texture, color, and weight), the highest fresh weight (1.83 and 1.99) gm and dry weight (0.207 and 0.233) mg of callus *Rosa damascene* and *Rosa hybirda*, respectively in dark conditions compared with least in blue light.

Keywords: light quality, Tissue culture, *In vitro*, *Rosa damascene*, and *Rosa hybirda*.

استجابة نوعين من نبات Rosa لنوعية الضوء خارج الجسم الحي

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الخلاصة :

في هذه الدراسة ، تم تطبيق جودة الضوء الظلام والمتنوع (الأبيض والأحمر والأخضر والأزرق) لتقييم تأثيرها على خصائص النمو والمحتوى الكيميائي وخصائص الكالس لنباتي .*Rosa damascene* Mill و *Rosa hybirda* L. .

الجزء النباتي (العقدة المفردة والقمة النامية) زرعت على وسط MS المضاف إليه السكروز والأكار ومنظمات نمو النباتية (الكاينتين بتركيز 0.5 ملغم / لتر والاندول -3- بيوتريك اسيد بتركيز 1 ملغم / لتر لتجربة تكوين النبات الكامل أو 1 ملغم / لتر من الكاينتين مع 0.5 مجم / لتر الاندول -3- بيوتريك اسيد لتجربة الكالس) ، تم حضنها في غرفة النمو لثلاثين يومًا.

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أظهرت نتائج التجربة لتكوين النبات الكامل تباين في صفات النمو لنوعي ورد الجوري ، تسبب الضوء الأخضر والأبيض في ارتفاع نسبة النمو الخضري مقارنة بالظلام ، بينما تسبب الضوء الأحمر والأخضر في ارتفاع نسبة النمو الجذري مقارنة بالظلام . يحتوي النبات النامي باللون الأبيض أو الأخضر على نسبة أعلى من الكلوروفيل ، بالإضافة إلى الكربوهيدرات والبروتينات في الأجزاء الجذرية والخضرية مقارنة بالظلام.
أوضحت نتائج تجربة الكالس تباين في خصائص الكالس (الشكل والنسجة واللون والوزن) ، أعلى وزن طري بلغ (1.83 جم) والوزن الجاف (0.207 مجم) للكالس *Rosa hybirda* and *Rosa damascene* على التوالي النامي الظلام مقارنه باقلها بالضوء الازرق .

1. Introduction :

In the world, Rosas are among the most familiar and popular flowers [1]. Which may be distributed in Asia, Europe, ~~the~~ North, and Middle East America. For their flowers and fruits, these deciduous shrubs are often cultivated in gardens [2]. Subtropical regions of the Northern Hemisphere are home to more than 100 species of the genus Rosa [3]. A woody perennial belonging to Rosaceae [4]. Which has more than 18000 cultivars and 200 different species [5].

One of the most important ornamentals plants are Rosas, which are most often used for aromatic and medicinal purposes [6]. Traditional uses for the Rosa flower extracts include an astringent stomachic, agent for promoting blood flow and reducing blood stasis, therefore regulating menstrual, antitoxin, treating mastitis and diabetes. also used to preparing of Rosa tea extracts which are widely regarded as cosmetic and dietary products [1]. Rosa has a long history of usage in China as a food and medicine. Rosa petals have been used in teas, cakes, and flavoring extracts [4]. According to [7], leaves, roots, flowers and fruits of Rosa contain chemical components, ~~a~~ rich sources of chemical compounds that are both primary and secondary metabolites.

Seeds, cuttings, layering, and grafting are all viable methods for growing roses. While these techniques of rose propagation are laborious and time-consuming, seed propagation frequently yields variety. Therefore, it is necessary to develop effective techniques for the quicker growth of roses [8]. In more recent years, procedures for tissue culture have been used to regulate and commercially propagate roses [9].

Plants' growth *in vitro* depends entirely upon artificial light sources as an alternative to solar energy, which should provide light in the fitting regions of the electromagnetic range for photomorphogenic reactions and photosynthetic metabolism. Regulating light quality, photoperiod, also irradiances enable the production of desired characteristics of plants. Light conditions are important environmental factors that significantly affect plant growth and development. Light quality (light source), quantity (intensity), and duration (photoperiod) are three principal characteristics that affect plant growth and functional component formation through photosynthesis.

Light is the most important environmental factor affecting ~~on~~ photosynthesis therefore, the plant grows better [10]. Light quality regulates cell metabolism, morphogenesis, gene expression, and other physiological processes [11]. Plant growth is affected by the interaction between the level of endogenous plant hormones and light quality [12]. Therefore, this research aims to choose the optimal light source and its effects on the growth characteristics, chemical content, and callus characteristics of *Rosa damascene* Mill. *and Rosa hybirda* L.

2. Materials and methods :

Experiments were carried out in a tissue culture lab. in biology department - science collage - Al-Qadisyah university during the period from August 2021 to June 2022 to study the effect of Dark and various lights quality: white light (W, 420nm), blue (B, 460nm) green (G, 560nm) and red (R, 660nm) on growth characteristics, chemical content of shoot and root, and callus characteristics of *Rosa damascene* Mill. and *Rosa hybirda* L. using tissue culture technique.

- Sterilization of Explant

Explants (single-node and shoot tips) washed under running tap water to remove adherent soil, then remove the leaves, then spry ethyl alcohol 70% for 1 minute and soak for 20 min in commercial bleach NaOCl (8%) containing 2 drops Tween-20 to aid wetting. Finally, in A laminar flow cabinet, the explant was washed with sterilized distilled water 3 times for 5 minutes to remove any side effects of sterilization and maintain ~~the~~ vitality [13].

- Preparation MS medium

Media for Rosa cultures contained 4.44 gm/l Murashige & Skoog media, 30 gm/l sucrose, then add growth regulators 0.5 mg/l kinten and 1 mg/l butyric acid for whole plant experiment or 1 mg/l kinten and 0.5 mg/l Indole butyric acid for callus experiment. The pH of MS media was adjusted at 5.7 $^{\pm}$ 1 using HCL (0.1 N) or NaOH (0.1 N) before adding 7 gm/L agar and using a hot plate magnetic stirrer to homogenize the components of the medium at a temperature of 70 °C for 15 minutes and then distributed in sterile culture jars (35 ml capacity) each containing 6 ml of medium. after that medium was sterilized by Autoclave at 121°C and 1.05 kg/cm2 (15-20 psi) for 20 minutes.

- Culture Explant

Sterilized explant (single-node or apical meristem) 0.5 cm long culture on MS medium in A laminar flow cabinet, and incubated in the growth room. At a temp. of 25$^{\pm}$1 °C under Dark and various lights (white, red, green, and blue) with 16/8-hour photoperiod (switch on/off). For about 30 days, cultures were maintained in the same media and then transferred into the same new media.

The results were taken after 4 weeks. At the end of the incubated period the growth parameters were calculated as the mean number of the highest rate of length shoot and root, the number of branches, leaves and roots, fresh and dry weight of shoot and root, and shoot content of chlorophyll a, b and total. Shoots and roots content of carbohydrates and protein. The characteristics of the callus (color, texture, and shape) and fresh and dry weight of the callus [14].

- Experimental design :

Data analysis was carried out using a complete randomized design (CRD) in a factorial arrangement 2×5 (Rosa type and light quality) with 10 replications per treatment. When the treatment effect was significant, Revised Least Significant Difference (RLSD) was applied to compare averages at a probability **0.05** level [15].

3. Results :

The statistical analysis of ~~The~~ Table (1) below shows that the lighting factor has a significant effect on all the growth characteristics of Rosa, where white light has the highest rate of emergence explant in vitro 100% for both types of plants compared to other treatments.

As for the growth indicators of explant, the highest rate of length shoot, number of branches and leave, fresh and dry weight of shoot under green light conditions, followed by those who were exposed to white light, Figure(1). While the highest rate of length root, number of branches of root and fresh and dry weight of root under the conditions of red light then green, Figure(2).

Table 1: Showing the effect of light conditions in the Characteristics studied of two types of Rosa after 4 weeks of culture.

type of Rosa	Light conditions	Emergence explant after 30 day	Length of (cm)		Number of (Per plant)			Fresh weight of (gm)		Dry weight of (gm)	
			shoot	root	leave	branch	root	shoot	root	shoot	root
Rosa damascena	White	100	7.24	2.44	6.78	1.78	1.08	3.18	0.72	0.333	0.084
	Dark	91	2.91	1.09	0.97	0.67	0.81	2.61	0.60	0.269	0.061
	Green	95	7.93	2.97	8.76	2.06	1.34	3.32	0.87	0.341	0.093
	Red	90	4.61	3.14	5.22	1.92	1.81	2.93	0.92	0.309	0.104
	Blue	81	3.92	1.32	3.68	1.88	0.93	2.48	0.67	0.264	0.025
Rosa hybirda	White	100	6.88	2.01	4.42	4.42	1.02	3.34	0.81	0.373	0.091
	Dark	95	2.07	0.93	0.61	0.61	0.89	2.81	0.57	0.274	0.059
	Green	96	7.09	3.07	6.06	6.06	1.06	3.82	0.97	0.379	0.101
	Red	92	3.91	3.44	3.29	3.29	1.29	3.01	0.98	0.332	0.132
	Blue	83	3.62	1.84	1.91	1.98	0.94	2.51	0.75	0.242	0.067
R.L.S.D. at 0.05		4.45	1.95	1.07	2.09	0.94	0.61	0.34	0.11	0.088	0.021

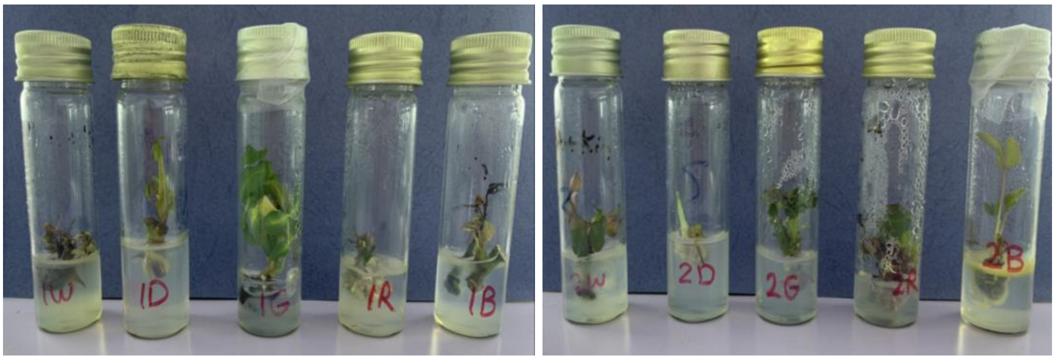

Figure 1: Shoot growth of Rosa *invitro* under different light quality (1: *Rosa damascena* , 2: *Rosa hybrid*) – (W: white, D: dark, G: green, R: red, and B: blue light)

Figure 2: Root growth of Rosa *invitro* under different light quality (1: *Rosa damascena* , 2: *Rosa hybrid*) – (W: white, D: dark, G: green, R: red, and B: blue light)

The results in Table 2 indicate explant for both types of plants growing in white light has a higher content of photosynthetic pigments: chlorophyll a, chlorophyll b, and total chlorophyll, as well as produced carbohydrates and proteins in shoot and root parts compared to the least in explant growing in dark.

Table 2: Showing the effect of light conditions on chemical contents of two types of Rosa after 4 weeks of culture.

type of Rosa	Light conditions	Pigment content in the shoot			Carbohydrate		Protein	
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	shoot	root	shoot	root
Rosa damascena	White	1.27	1.12	2.76	0.56	0.47	0.42	0.28
	Dark	0.24	0.35	0.97	0.12	0.19	0.21	0.17
	Green	1.24	1.09	2.53	0.29	0.32	0.32	0.26
	Red	0.73	0.81	1.67	0.22	0.24	0.29	0.22
	Blue	0.63	0.79	1.51	0.17	0.25	0.24	0.18
Rosa hybirda	White	1.13	0.91	2.45	0.43	0.39	0.51	0.35
	Dark	0.35	0.32	0.72	0.22	0.14	0.26	0.21
	Green	1.09	0.99	2.21	0.27	0.17	0.37	0.29
	Red	0.62	0.53	1.34	0.31	0.21	0.35	0.24
	Blue	0.49	0.39	1.21	0.20	0.19	0.34	0.19
R.L.S.D. at $_{0.05}$		0.18	0.09	0.12	0.038	0.041	0.047	0.035

The results of Table (3) explained significant differences in the characteristics of the callus (color, texture, and shape) under different lighting conditions Figure(3). The optimal conditions for the highest fresh and dry weight of callus reached in explant growing in dark or white light and lowest in blue light where callus grew slowly and became more compact and yellowish green or whiteish green. The highest fresh (1.83gm) and dry weight (0.207mg) of *Rosa damascena* callus in dark conditions, while the highest fresh (1.99gm) and dry weight (0.223mg) of *Rosa hybirda* callus also in dark conditions, with significant differences among all treatments.

Table 3: Showing the effect of light conditions on the callus Characteristics for two types of Rosa after 4 weeks of culture.

type of Rosa	Light conditions	Callus Characteristics			Callus Weight	
		color	texture	shape	fresh	dry
Rosa damascena	White	brown	friable	granular	1.73	0.181
	Dark	pure white	friable	granular	1.83	0.207
	Green	Green	Semi Compact	Wavy	1.43	0.149
	Red	Whiteish green	Compact	compressed	1.41	0.141
	Blue	Yellowish Green	Compact	compressed	1.13	0.124
Rosa hybirda	White	Whiteish yellow	friable	granular	1.83	0.198
	Dark	Whiteish green	friable	granular	1.99	0.223

	Green	Green	friable	granular	1.61	0.153
	Red	Greenish Yellow	Compact	compressed	1.53	0.156
	Blue	Whiteish green	Compact	compressed	1.24	0.131
R.L.S.D. at $_{0.05}$		-	-	-	0.18	0.091

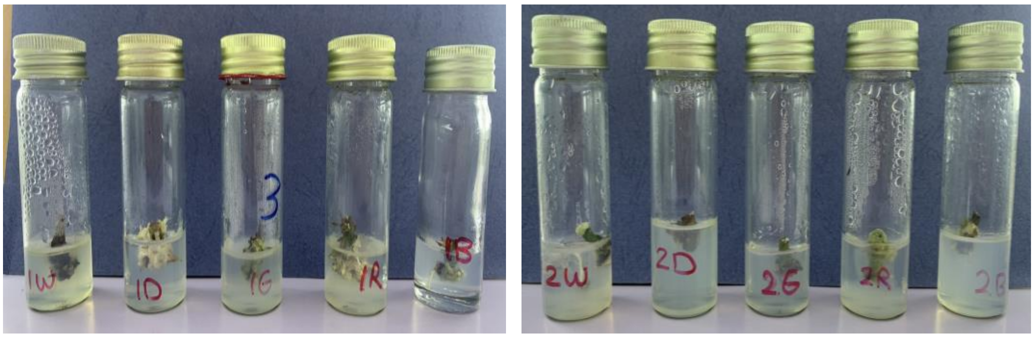

Figure 3: Callus growth of Rosa *invitro* under different light quality (1: *Rosa damascena* , 2: *Rosa hybrid*) - (W: white, D: dark, G: green, R: red, and B: blue light)

4. Discussion :

Light spectra have different wavelengths, photosynthetically active radiation (PAR) which falls between 400 nm to 700 nm, the most crucial wavelength for plant growth [16]. Therefore, of the results achieved, the causes for different growth indicators by light conditions are due to the physiological responses of plants, and the process of metabolism, which is reflected in the growth of shoot and root. also pointed out that light is an environmental factor that has a significant influence on increasing the ability to absorb plant hormones, especially cytokines by explant. It also reduces the side effects by producing high concentrations of auxin and cytokines added to the medium. Thus, choosing the optimal light quality was significant for the development explant [17].

The light quality or intensity-dependent changes in morphogenesis and plant physiology are regulated by phytohormones [18] [19]. As a result the interaction between light signals from the environment and biosynthesis of endogenous phytohormones auxin and cytokinin, which directly or indirectly affect the growth of shoot and root [20]. Red light induces chlorophyll biosynthesis so plants can photosynthesis, also blue light for plant morphology [21].

The ratio of red light is four times more than blue light perhaps to encourage the plant to photosynthesis and induce plant growth, which agrees with the results reported by [22] where light quality will be induced or inhibited of the shoot and root growth. According to, [23] red light to induce the photosynthesis process has been accepted extensively. [24] explain red wavelength was competent to be absorbed by photosynthetic pigments. Moreover, the optimum absorption peak of chlorophyll was red. but Blue light has significant roles in plants including the stomatal process, gas exchange, and water relations [25]. Therefore, red and blue light may be useful for photosynthesis ~~in~~ and thus for the growth and development of shoots and roots [26].

The plant reflects green light, therefore plants appear green and have been thought to be of no use for plant growth by decreasing photosynthesis processes. But plant exposure to green light induces stem elongation, which means useful [27]. Although the rate of absorption of red light or blue by leaves is about 90% but the green light is about 75% [28]. Greenlight also affects plant physiology and morphology, including leaf growth, early stem elongation, and stomatal conductance [27] [22]. Generally, callus with active growth and friable structure was easy to dedifferentiate, and the light intensity had a greater influence on the texture, color, shape, and physiological state of the callus, where the reason for this induced callus growth may be natural auxin levels increased under darkness or lower light intensity [29]. As result, the oxidation processes of phenolic compounds induced by light exposure [30] [31], therefore, the callus showed a higher browning rate and darker browning color under a higher light intensity which was reflected in the growth and development of callus and explants *in vitro*.

Conclusion :

Depending on the results of our research, I note that exposing the explant to the green light plays an important role in increasing many indicators of shoot growth, while the red light recorded the best results of root growth. As for the characteristics of the callus, it was better when incubated explant in the dark.

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