Effects of Light Spectrum on the Growth, Photosynthetic Pigments and some Stomata Characteristics of Broad Bean (*Vicia faba* L.)

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***Abstract*—A study with complete randomized design with five replications was conducted in Koya city during winter season 2016–2017 to study the effects of four light spectrums (white, blue, green, and red) in addition to the control (plants grow under sunlight) on some vegetative growth, photosynthetic pigments, and stomata characteristics of *Vicia faba* L. cv. Franchi. The results showed that plants grown under green light increased plant high significantly to 49.67 cm compared to other treatments, whereas the lowest value was 14.33 cm which recorded in control treatment. Highest nodes and leaves number were recorded in plants of white light treatment (6.00 nodes/plant and 4.67 leaves/plant), whereas the lowest number recorded in the plants grown under red light (3.33 nodes/plant and 2.67 leaves/plant). Leaf area of the control plants increased significantly to 195.75 cm^3 compared to other treatments. Root length increased significantly to 25 cm in plants exposed to blue light compared to other treatments except for the red light (22.27 cm). The lowest amount of chlorophyll a and b and higher content of total carotenoids (15.78, 9.44, and 3.12 mg/g fresh weight) were recorded in plants exposed to green light significantly compared to other treatments, whereas the highest chlorophyll a (18.38 mg/g fresh weight) was recorded in white light treatment, and chlorophyll b (20.40 mg/g fresh weight) in control plants which also gave the highest amount of total carotenoids (0.54 mg/g fresh weight). Stomata number increased significantly in plants exposed to red light to 76.67 and 120.00 stomata/mm^2 for adaxial and abaxial leaves surfaces, respectively, whereas lowest number was recorded in plants exposed to green light (53.33 stomata/mm^2).**

***Index Terms*—Light spectrum, Photosynthesis pigments, Stomata characteristics, Vegetative growth, *Vicia faba* L.**

I. Introduction

The broad bean or fava bean (*Vicia faba* L.) is a member of the vetch family (Fabaceae), grows in temperate regions. Broad bean is a famous winter leguminous crops in Iraq, their seeds contain 51–68% total carbohydrates and 28–30% protein, it is considered as a good source of vegetarian protein for human consumption, and it is cultivated in crop rotations to improve soil properties [1,2].

Light plays a key role in plant life, determining their photomorphogenesis and photosynthesis rates [3]. Radiant energy from the sun consists of many different wavelengths of light. Only photons of wavelengths from 400 to 700 nm are utilized in photosynthesis, and about 85–90% of this photosynthetic active radiation are absorbed by the leaf; the remainder is either reflected at the leaf surface or transmitted through the leaf [4]. As electrons in molecules can have only discrete energy levels, only photons that provide a quantity of energy adequate for an electron to "jump" to another possible energetic state can be absorbed. The consequence of this is that substances have colors and they absorb photons with only certain energies. Light signals can regulate changes in structure and form, such as seed germination, leaf expansion, stem elongation, flower initiation, and pigment synthesis [5]. Light spectral composition reaching plants affects their growth and development through the participation of plant photoreceptor cells [6]. Cryptochromes and phototropins are specifically blue light-sensitive, whereas phytochromes are more sensitive to red than to blue light [7]. Red light is important for the development of the photosynthetic apparatus of plants and may increase starch accumulation in several plant species by inhibiting the translocation of photosynthates out of leaves [8]. Blue light is important in the formation of chlorophyll, chloroplast development, stomatal opening, and enzyme synthesis [9]. When white light enters the upper surface of a leaf, blue and red photons are preferentially absorbed by chloroplasts near the irradiated surface, due to the strong absorption bands of chlorophyll in the blue and red regions of the spectrum. Greenlight, on the other hand, penetrates deeper into the leaf. Compared to blue and red, the most widespread group of pigmented flavonoids is the anthocyanins, which are responsible for most of the red, pink, purple, and blue colors observed in plant parts [4].

Many studies were conducted on the effects of light on plant growth; different literatures supply varying information on the effect of light spectrum on the plant growth process including each of shoot and root and physiological

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characteristics, where they considerably depend on the species. For example, for gerbera (*Gerbera aurantiaca* L.) blue light stimulates root elongation, while red light inhibits it [10]. Different results were recorded by Kozak [11] for common gardenia (*Gardenia* sp.), where blue light inhibited root elongation significantly, while white light stimulated it, and the highest fresh weight of shoots was obtained under red light. According to Głowacka [12] in case of tomato seedlings, a high fresh and dry matter was obtained under blue light. Lin *et al*. [13] stated that the shoot and root fresh and dry weights of lettuce (*Lactuca sativa* L. var. capitata) plants treated with red-blue (RB)-white and fluorescent (FL) lights were higher than those of plants treated with RB lights, however, the chlorophylls and carotenoids of lettuce leaves showed no significant differences among treatments. Fraszczak [9] studied the effects of the end-of-day and the end-of-night red and blue light in the growth of dill (*Anethum graveolens* L.) cv. Ambrozja which exposed to white diode light. The red and blue light was employed for 30 min before the initiation or after the end of the lighting period. The values of plant fresh mass, area and height parameters were the highest for plants treated with red light at the end of the night. Plants treated with blue light at the end of the lighting period were characterized by the poorest growth rate. The study of Schroeter-Zakrzewska and Kleiber [14] shows a significant increase in fresh weight of the cutting of Michaelmas daisy (*Aster amellus* L.) grown under red and blue colors, whereas the differences did not significant on dry weight.

The objective of this study was to investigate the impact of a different light spectrum on some growth, photosynthesis pigments, and stomata characteristics of broad bean plants.

II. Materials and Methods

A. Plant Material and Treatments

Under laboratory environment a complete randomized design with 5 replicates was conducted by using compact fluorescent light bulbs with four spectrums including white, blue, green, and red in addition to the control treatment where the plants growing under sunlight, to study their effects on broad bean (*V. faba* L.) cv. Franchi, which their seeds were cultivated in plastic pots on the December 6, 2016. Temperature, relative humidity, and light intensity during the study period are appearing in Table I. On the February 20, 2017, the following characteristics were studies: Shoot length, number of plant nods and leaves, and plant leaf area which calculated by the method described by Watson and Watson [15], stem diameter was measured using micro vernier, percent of shoot and root dry matter, main root length, chlorophylls, and total carotenoids were estimated according to Lichtenthale and Wellburn [16] and number, length, and width of stomata in the adaxial and abaxial leaves surfaces which measured by the method of lasting impressions as it described by Rai and Mishra [17].

TABLE I
Average Values of Temperature, Relative Humidity, and Light Density During Plants Growing

Property	Light treatments				
	Control	White	Blue	Green	Red
Temperature (C°)	20.03	17.2	17.7	17.3	16.9
Relative humidity (%)	51.0	49.2	52.2	52.8	49.0
Light intensity (K. Lux)	52.80	1.7	0.35	0.50	0.70

B. Statistical Analysis

Data subjected to analysis of variance using SAS program. Treatments means were compared using Duncan's Multiple Range test ($\alpha = 0.05$) [18].

III. The Results

The results of Table II and Fig. 1 show that plants grown under green light increased shoot length to 49.67 cm significantly compared to other treatments, the stem was thin and the plants appeared to droop, and the stem was not strong enough to hold it. Lowest shoot length was for control treatment which records 14.33 cm. Highest nodes number recorded for white light plants which gave 6.00 nods/plant, whereas the lowest (3.33 nods/plant) recorded for the plants of red light treatments. Highest plant leaves number (4.67 leaves/plant) was recorded for plants exposed to white and green lights, respectively, whereas the lowest value recorded for the red light (2.67 leaves/plant). Plant leaf area of control treatment was the highest (195.75 cm^2) significantly compared to other treatments, followed by the white light (115.68 cm^2), whereas each of green, blue, and red lights decrease the leaf area significantly to 46.83, 37.60, and 28.54 cm^2, respectively. The results in Table I also reveal with non-significant differences between light spectrums on vegetative growth characteristics except the decrease in stem diameter for plants grown under red light (4.08 mm) significantly compared to control, white and green treatments. Highest percent of shoot dry matter was recorded in plants grow in control condition (8.80%) significantly compared to red and white lights (7.25 and 6.66%), respectively.

TABLE II
Effects of Light Spectrum on some of Vegetative Growth Characteristics of Broad Bean

Light spectrum	Shoot length (cm)	Nodes number/plant	Leaves number/plant	Plant leaf area (cm^2)	Stem diameter (mm)	Shoot dry matter (%)
Control	14.33^c	4.67^b	4.33^a	195.75^a	5.50^a	8.80^a
White	40.00^b	6.00^a	4.67^a	115.68^b	5.50^a	6.66^b
Blue	21.67^c	5.33^{ab}	3.33^{ab}	37.60^c	5.10^{ab}	7.76^{ab}
Green	49.67^a	5.33^{ab}	4.67^a	46.83^c	5.37^a	7.45^{ab}
Red	19.33^c	3.33^c	2.67^b	28.54^c	4.08^b	7.25^b

Means followed by the same letters within a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range test

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Results in Table III indicate that root length increased significantly in plants exposed to blue light (25.00 cm) compared to other treatments except that exposed to red light (22.27 cm), whereas green light decreased root length to 18.33 cm. In respect to percent of root dry matter, the white light decreased this percent significantly to 3.78% compared to other treatments, whereas highest percent recorded in plants exposed to red light (7.68%).

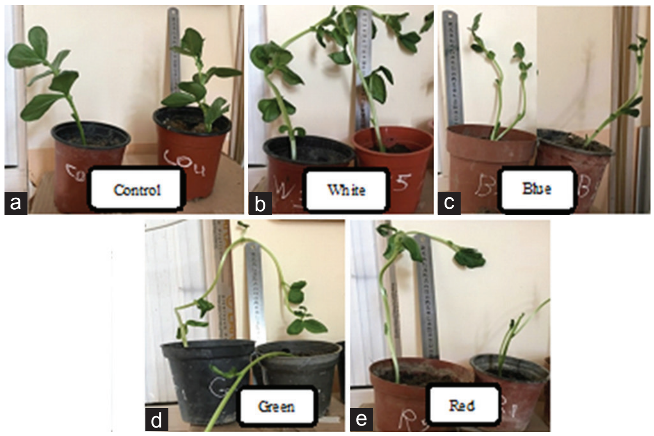

Fig. 1. *Vicia faba* L. plants grown under different spectrums of fluorescent light. (a) Control, (b) white (c)blue, (d) green, (e) red.

TABLE III
Effects of Light Spectrum on some of Roots Characteristics of Broad Bean

Light spectrum	Root length (cm)	Root dry matter (%)
Control	21.17^{bc}	6.57^{a}
White	21.00^{bc}	3.78^{b}
Blue	25.00^{a}	5.72^{a}
Green	18.33^{c}	7.42^{a}
Red	22.27^{ab}	7.68^{a}

Means followed by the same letters within a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range test

TABLE IV
Effect of Light Spectrum on some Photosynthesis Pigments in Broad Bean Leaves

Light spectrum	Chlorophyll a	Chlorophyll b	Total carotenoids
	(Mg/g fresh weight)		
Control	17.37^{a}	20.40^{a}	0.54^{b}
White	18.38^{a}	20.18^{a}	0.66^{b}
Blue	17.44^{a}	17.46^{a}	1.33^{b}
Green	15.78^{b}	9.44^{b}	3.12^{a}
Red	17.5^{a}	19.41^{a}	0.87^{b}

Means followed by the same letters within a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range test

The results shown in Table IV appear that lowest amount of chlorophyll a and b (15.78 and 9.44 mg/g fresh weight) was recorded in leaves of plants exposed to green light significantly compared to other treatments, whereas the highest chlorophyll a (18.38 mg/g fresh weight) was recorded in plants exposed to white light, and chlorophyll b (20.40 mg/g fresh weight) was recorded in control plants. The effects of light spectrum on leaves content of total carotenoids were the opposite of that of chlorophyll a and b, where the green light records the highest value of total carotenoids (3.12 mg/g fresh weight) significantly compared to other treatments, whereas the lowest value recorded in the leaves of control plants.

Regarding the effects of light spectrum on stomata number, the results appear in Table V and Figs. 2 and 3 show that stomata number increased significantly to 76.67 and 120.00 stomata/mm^2 for adaxial and abaxial leaves surfaces, respectively, in plants exposed to red light, whereas lowest number recorded in plants exposed to green light (53.33 stomata/mm^2) for both leaves surfaces. Stomata length in adaxial surface increased to 19.33 µm in plants exposed to white light significantly compared to plants exposed to green light (13.67 µm), whereas for abaxial surface highest stomata length was recorded in plants exposed to red light (18.67 µm) significantly compared only to plants exposed to blue light which gave the lowest value (14.33 µm). In respect to stomata width, there were non-significant differences between different light spectrums on stomata width on adaxial surface, whereas, on abaxial surface widest stomata (10.00 and 9.33 µm) were recorded for the plants exposed to red light and the control treatments compared only to that exposed to white light (6.00 µm).

It is clear from the previous results that most effects were due to the differences in the light spectrum, not the temperature or relative humidity (Table I), which were very close to each other, in contrast to the wide range of light spectrums especially between the control and other treatments.

IV. Discussion

Increasing the length of plants that exposed to green light (Table II and Fig. 1) theoretically is close to the behavior of that grow in dark, because the light will reflect due to the

TABLE V
Effects of Light Spectrum on some of Stomata Characteristics on Adaxial and Abaxial Surfaces of Broad Bean

Light spectrum	Adaxial surface			Abaxial surface		
	Number of stomata/mm^2	Stomata length (µm)	Stomata width (µm)	Number of stomata/mm^2	Stomata length (µm)	Stomata width (µm)
Control	63.33^{ab}	17.00^{a}	8.33^{a}	86.67^{ab}	17.00^{ab}	9.33^{a}
White	66.67^{ab}	19.33^{a}	10.33^{a}	86.67^{ab}	15.67^{ab}	6.00^{b}
Blue	70.00^{ab}	17.33^{a}	9.67^{a}	70.00^{b}	14.33^{b}	7.67^{ab}
Green	53.33^{b}	13.67^{b}	8.33^{a}	53.33^{b}	16.33^{ab}	8.67^{ab}
Red	76.67^{a}	16.33^{ab}	8.67^{a}	120.00^{a}	18.67^{a}	10.00^{a}

Means followed by the same letters within a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range test

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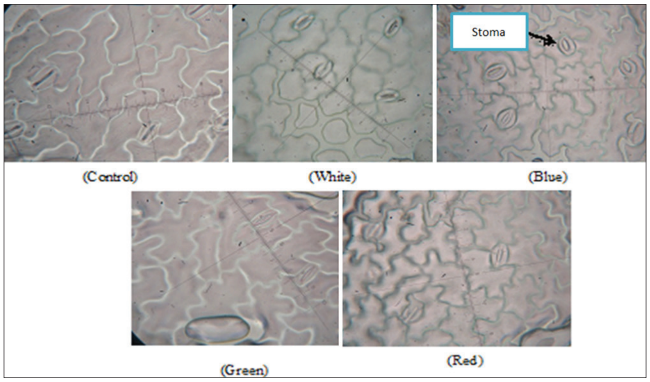

Fig. 2. Detail of ×400 microscopic observation of adaxial surface stomata in a *Vicia faba* L. plants leaves grown under the different fluorescent light.

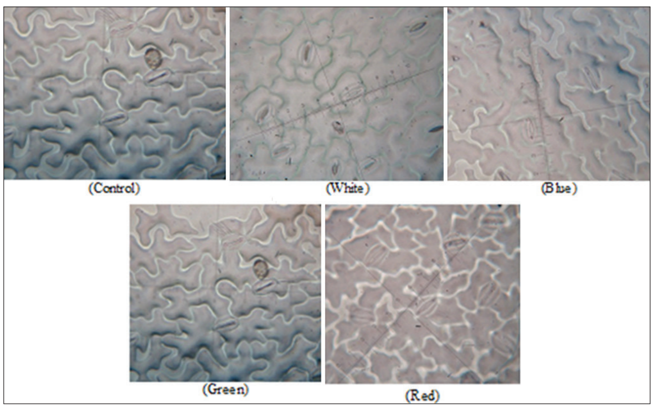

Fig. 3. Detail of ×400 microscopic observation of abaxial surface stomata in a *Vicia faba* L. plants leaves grown under different fluorescent lights.

greenness of the plants, where the effective wavelength that influences plant vegetative growth is ranged between 400 and 520 nm which is include green, violet, and blue bands [19]. The decrease in most vegetative characteristics for plants grown under red and blue lights may due to the phytochrome, a protein pigment that absorbs red and far-red light most strongly, but that also absorbs blue light, phytochrome play a key role in light-regulated vegetative and reproductive development, whereas blue light inhibits the hypocotyl elongation and enhance respiration. In fact, the 400–500 nm blue region of the action spectrum for the inhibition of stem elongation closely resembles that of phototropism, where the inhibition of stem elongation, perception of blue light depolarizes the membrane potential of elongating cells, and the rate of elongation rapidly decreases [4,20]. In general, the magnitude of the response to far-red light depends on the species, developmental stage, and other environmental variables such as blue light and/or photosynthetically active irradiance incident on the leaves [5]. Our results agreed with Yang *et al.* [21] who found that ending of the day with red (R, 600–700 nm wavelength) and far-red (FR, 700–800 nm) lights can reduce and enhance the stem/hypocotyl elongation rate, respectively.

Colors that do not have a high wavelength have a high level of energy; thus, plants in white spectrum had the highest tendency to generate more cells in the foliage of beans [22]. Our results agreed with Acero [23] who stated that white spectrum gave longest *Brassica rapa* plants where exposed to white, blue, green, yellow, and red FL light spectrums for 14 days of exposing, whereas the red spectrum gave the shortest plants.

Light quality incident on shoots can affect the growth of roots by indirectly affecting the photosynthate availability in roots, either because of changes in allocation or changes in assimilation. In turn, changes in the root growth, morphology and symbioses can affect the ability of plants to take up nutrients from the soil. More directly, light quality can affect the metabolism of enzymes involved in nitrogen metabolism. In some cases decreased R: FR has decreased root growth compared to shoot growth [5], the same effect may cause the increase in root length for plants grown under blue and red lights, as is shown in Table III.

To convert the chlorophyll precursor protochlorophyllide to chlorophyllide, light is required [24], so exist of lights is necessary; therefore, synthesis of chlorophyll pigment was not affected significantly by different light qualities (Table IV), except the green light where low chlorophylls were synthesis and increase in total carotenoids because chloroplasts absorb light mainly in the red and blue parts of the spectrum, so only some of the light enriched in green wavelengths (about 550 nm) [4]. The results also affirm that the effects of green light tend to reverse the processes established by red and/or blue light. In this way, green light may be functioning in a manner similar to FR light, informing the plant of photosynthetically unfavorable conditions.

These findings remind us that nature tends not to ignore a conditional environmental input and that inductive biological systems often have antagonistic systems that counter their progression. In this way, plants use the full spectrum and the relative ratios of energies within to adjust their form, composition, and physiology to best exploit prevailing conditions [6], where green light sensory systems adjust development and growth in orchestration with red and blue sensors.

Light-stimulated stomatal opening in the epidermis of *V. faba* L. especially the blue light (Table V, Figs. 2 and 3), the blue light stimulated stomatal movements are driven by blue light-dependent changes in the osmoregulation of guard cells. Blue light stimulates an H^+-ATPase at the guard cell plasma membrane and the resulting pumping of protons across the membrane generates an electrochemical-potential gradient that provides a driving force for ion uptake. Blue light also stimulates starch degradation and malate biosynthesis. Solute accumulation within the guard cells leads to the stomatal opening. Guard cells also utilize sucrose as a major osmotically active solute, and light quality can change the activity of different osmoregulatory pathways that modulate stomatal movements [4]. The effects of red light were more than others in our study in broad beans, maybe because physiological responses to spectral changes can vary among different plant species [25].

According to the results in Figs. 2 and 3 that stomata of green light treatments were partially closed as it cleared by Frechilla *et al*. [26] who demonstrated if *V. faba* epidermal

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peels exposed to green light followed by blue light, the stomata opened, whereas if the pulsed sequence was green, blue, and green the stomata remained closed.

V. Conclusions

Depending on the results of this work, we concluded that different light spectrums have different response in broad bean plants growth, pigments, and stomata characteristics. Base on the results of the study, mono spectrum FL lights were not recommended as a source of lighting for *V. faba* L. plants, compared to plants grown in natural lighting conditions. More studies are recommended about the effects of combinations of the different light spectrum or alternative coloring on the plants, in addition to using light emitting diodes lamps which are used recently in wide scope in plant production.

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