The Role of Melatonin in Improving The Vegetative Growth Characteristics of Four Genotypes of Faba Bean (Vicia faba L.)

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Abstract. A field study was conducted at a research station in Al-Hamidiyah, which is affiliated to the College of Agriculture - University of Anbar, during winter 2020-2021. It aims to study the effect of four concentrations of the growth regulator melatonin on the vegetative growth traits of four genotypes of faba bean. A split-plot arrangement in randomized complete block design RCBD, in three replications, was used. The concentrations of the growth regulator occupied the main plots (0, 5, 10 and 15) mg.l\(^{-1}\). While the American (Sakis), Dutch (Aquadulce), Italian (Ackerbhone) and Turkish (Aquadlge) genotypes occupied the sub plots. The most important results were the following: The American genotype superiority in most growth traits gave the height of plant is 96.94 cm, branches number in plant is 10.08, leaf area is 4770 cm\(^2 \cdot \)plant\(^{-1}\), dry weight of plant is 184.71 g, chlorophyll content in the leaves is 48.21 spad, and the length of the pod is 19.63 cm. As for the Italian variety, it gave the lowest period from planting to the beginning of flowering, which was 59.42 day. As for the Turkish genotype record, the longest period from planting to beginning of flowering 64.57 day. Spraying with the growth regulator melatonin led to the emergence of significant differences between all the traits. The concentration 15 mg.l\(^{-1}\) of melatonin gave a highest mean for each: plant height is 78.23 cm, branches number is 8.50, leaf area is 3840 cm\(^2 \cdot \)plant\(^{-1}\), dry weight is 177.63 g, chlorophyll content in the leaves is 44.02 spad, and the length of the pod is 18.15 cm. There was a significant overlap between American genotype with concentration 15 mg.l\(^{-1}\) in each of the leaf area, dry weight of the plant, and chlorophyll content of leaves. The overlap was significant between American genotype with concentration 10 mg. l\(^{-1}\) in plant height.

1. Introduction

The faba bean (Vicia faba L.) is one of the crops of the legume family (Fabaceae), which occupies the second place after the cereal crops in terms of importance. The importance of this crop is that it is one of the important food sources for millions of people, especially in poor and low-income communities in East and North Africa and some Asian countries, as it is one of the sources rich in protein. The percentage of protein in its seeds ranges between 25-40%, and it also contains carbohydrates, vitamins and some mineral elements [1]; [2]. As well as using it as animal feed and as a green organic fertilizer in poor soils to improve its characteristics through its contributions to biological nitrogen fixation through root nodes in coexistence with Rhizobium bacteria, so it is used in agricultural rotations [3]. The genotypes of faba bean are sensitive to drought and high temperatures during flowering and pod formation [4]. The genotypes of the faba bean differ in the nature of their growth.
and morphology, as well as their impact and the extent of their response to the prevailing environmental factors. The use of plant growth regulators of both types encouraging and inhibiting, which can be considered as an agricultural tool that makes the plant use nutrients efficiently and exploit its physiological and genetic potential to the highest level [5]; [6]. Melatonin is one of the plant growth regulators that works to relieve abiotic stresses such as high and low temperatures, excessive salinity, drought and senescence [7]. It also participates in many physiological and regulatory processes in the plant, such as the formation of the main roots and the increase of their lateral branches [8]. It maintains the cellular oxidative balance by activating the antioxidant enzymes, protecting the chloroplasts and delaying the senescence of the leaves, which increases photosynthesis process and this is reflected positively to increase the growth of the crop [9].

The aims of this study to find out the effect of the growth regulator melatonin on the vegetative growth characteristics of faba bean crop.

2. Materials and Methods
A field study was conducted in Al-Hamidiyah research station, which is affiliated to the College of Agriculture - University of Anbar, during the winter 2020-2021. To study the effect of four concentrations of the growth regulator melatonin on the vegetative growth traits of four genotypes of faba bean. A split-plot arrangement in randomized complete block design (RCBD) with three replications was used. The concentrations of the growth regulator occupied the main plots (0, 5, 10 and 15) mg.l⁻¹. Each concentration of melatonin growth regulator was sprayed in the early morning until the leaves were completely wet, twice, the first at the beginning of flowering and the second at the beginning of the formation of the pods. While the comparison treatment was sprayed with water only. As for the American (Sakis), Dutch (Aquadulce), Italian (Ackerbhone) and Turkish (Aquadlge) genotypes occupied the sub plots. The experimental land was plowed by two orthogonal plows, and then soil service operations were conducted, then it was divided into experimental units. The dimensions of the experimental unit were 3 x 3 m and it contained four lines. The experiment was irrigated and then the seeds were planted on 10/10/2020, the irrigation process continued whenever needed. After emergence, the plants were thinned out, leaving only one plant in each place, to became the plant density 53,333 plants per hectare. The experiment was cleared of weeds as often as needed. Two batches of nitrogen fertilizer were given in the form of urea (N46%), the first at planting and the second at the beginning of flowering, with an amount of 80 kg. h⁻¹. As for the phosphate fertilizer, it was added all at once when planting in the form of triple superphosphate (46% P₂O₅) with an amount of 120 kg.h⁻¹[10]. The experiment was harvested on 1/4/2021. The Traits Studied were as follows.

2.1. The period from planting to the beginning of flowering (day):
was calculated at the beginning of flowers in plants for each experimental unit.

2.2. The height of Plant (cm):
The measurement was carried out from the soil surface to the top of the plant and as an average of five plants from each experimental unit at the full maturity of the crop.

2.3. branches number in the plant:
The average number of branches in the five plants was calculated.

2.4. Leaf area (cm²):
The leaf area of the plant was measured in the flowering stage as the mean of three leaves from each experimental unit, according to the following equation [11]

\[ LA = 0.04 + 0.45 \times (LW) \]

LA = the leaf area of the plant
L = leaflet length
W = width of the leaflet
Then the leaf area is multiplied by number of leaves per plant to calculate the total leaf area.

2.5. Dry weight of the plant (gm):
It was calculated as the mean of five plants taken at the end of the pod-filling phase and dried at 70 °C for 48 hours and then weighed.

2.6. Leaf content of chlorophyll (Spad):
The total chlorophyll content in the leaves was estimated by a chlorophyll meter type Minolta (Spad502) of Japanese origin as an average of three readings and for three leaves randomly, for five plants from each experimental unit after the completion of the flowering stage, extract the medium of the plant.

2.7. Pod length (cm):
It was measured as the mean length of ten pods that were randomly selected from each experimental unit.

2.8. Statistical analysis
The data was analyzed statistically for the studied traits according to the design used, using the Genstat program, and the averages were compared using the L.S.D. test at a level of significance of 5%.

3. Results and Discussion

3.1. The period from planting to the beginning of flowering (day).
The Table (1) showed that the Italian genotype was the earliest in flowering, as it recorded the lowest period from planting to the beginning of flowering, which amounted 59.42 days. While the Turkish genotype was lagging behind in this trait, as it recorded an average of 64.67 days. The reason for the variation of the varieties in period from planting to the beginning of flowering may be attributed to Variation in genetics of them, Which led to a different response to the surrounding environmental conditions, so it differed in this characteristic. This result agrees with the results of [12, 13, 14].

Melatonin concentrations significantly affected the period from planting to the beginning of flowering. As it is clear from the results of Table (1) that the high concentration of melatonin 15 mg.l⁻¹ achieved a lowest mean for this trait of 60.08 days. As for the comparison treatment, the highest period was recorded from planting to the beginning of flowering, which amounted to 63.42 days. This may be due to the fact that the high concentration of melatonin may have contributed to the building and growth of different parts of the plant faster, including the flowering organs, which led to an earlier in period from planting to flowering [15]. While there was no significant overlap in this trait.

| Genotypes (G)  | Concentration of melatonin mg.l⁻¹ (M) | Mean |
|----------------|-------------------------------------|------|
|                | 0   | 5   | 10  | 15  |       |
| American       | 64.33 | 62.00 | 61.00 | 59.00 | 61.58 |
| Dutch          | 62.67 | 62.00 | 61.00 | 59.00 | 61.17 |
| Italian        | 60.00 | 60.33 | 59.00 | 58.33 | 59.42 |
| Turkish        | 66.67 | 65.00 | 63.00 | 64.00 | 64.67 |
| Mean           | 63.42 | 62.33 | 61.00 | 60.08 |       |

L.S.D.0.05   | G = 1.065 | M = 1.016 | G X M = ns
3.2. plant height (cm)

The data in Table (2) shows the superiority of the American genotype over the rest of the genotypes in plant height, as it gave the highest average of 96.94 cm, followed by the Dutch and Italian genotypes, which recorded a mean of 84.33 and 72.97 cm, respectively. While the Turkish genotype recorded a lowest mean for the height of plant is 54.77 cm. The reason for the superiority of the American genotype in this trait may be attributed to its genetic nature in increasing the average division and elongation of cells, which positively affects the increase in plant height. These results agree with the findings of [16,17], which shows significant differences between the genotypes in plant height trait. As for melatonin concentrations, it had no significant effect on plant height.

The effect of overlap between genotypes and melatonin significantly on plant height. The American genotype with 10 mg.L\(^{-1}\) concentration of melatonin gave the highest mean of overlap in the height of plant trait of 97.57 cm. While the Turkish genotype with the comparison treatment gave the lowest mean in this trait, 53.63 cm.

### Table 2. The role of melatonin and genotypes and the overlap in height of plant (cm)

| Genotypes (G) | Concentration of melatonin mg.L\(^{-1}\) (M) | Mean |
|---------------|---------------------------------------------|------|
|               | 0                                          | 5    | 10   | 15   |      |
| American      | 97.30                                      | 95.51| 97.57| 97.37| 96.94|
| Dutch         | 82.97                                      | 84.50| 85.10| 84.77| 84.33|
| Italian       | 70.43                                      | 74.20| 72.43| 74.90| 72.97|
| Turkish       | 53.63                                      | 54.67| 54.80| 56.00| 54.77|
| Mean          | 76.08                                      | 77.22| 77.47| 78.23|      |

L.S.D.0.05 G = 1.029 M = ns G X M = 2.315

3.3. Branches number in the plant

Table 3 shows the superiority of the American genotype in the branches number in plant, giving the highest mean 10.08 branch plant\(^{-1}\). As for the Turkish genotype recorded the lowest mean 5.25 branch plant\(^{-1}\). The reason for the variation in genotypes of faba bean in branches number in the plant may be due to the genetic nature of these varieties in exploiting the growth factors and their response to the surrounding environmental factors. This result is consistent with what was stated by [17,18], which indicated that the genotypes of faba bean differ among themselves in the number of branches in the plant.

The results of the same table also indicate that spraying with melatonin led to a significant increase in the number of branches in the plant by increasing the concentrations. The concentration 15 mg.L\(^{-1}\) gave the highest average number of branches in the plant reached 8.50 branch plant\(^{-1}\). While the comparison treatment recorded the lowest average for this trait, which was 6.92 branches. Plant\(^{-1}\). This may be due to the role of melatonin in stimulating the lateral meristematic tissues, which increased the number of branches [8].

While there was no significant overlap in this trait.
Table 3. The role of melatonin and genotypes and overlap in branches number of plant.

| Genotypes | Concentration of melatonin mg.l⁻¹ | Mean |
|-----------|----------------------------------|------|
|           | 0                      | 5    | 10  | 15  |      |
| American  | 9.33                  | 10.00| 10.00| 11.00| 10.08|
| Dutch     | 7.33                  | 8.00 | 8.00 | 9.00 | 8.08 |
| Italian   | 6.33                  | 6.00 | 6.67 | 8.00 | 6.75 |
| Turkish   | 4.67                  | 5.33 | 5.33 | 6.00 | 5.25 |
| Mean      | 6.92                  | 7.25 | 7.50 | 8.50 |      |

L.S.D. 0.05  G = 0.874  M = 0.877  G X M = ns

3.4. Leaf area (cm²)
The genotypes significantly affected on the leaf area trait (Table 4). The American genotype superiority on the rest of the cultivars and gave the highest average for this trait amounting to 4770 cm². While the Turkish genotype record the lower mean of leaf area 3172 cm². It may be due to superiority of the American genotype in the trait of plant height (Table 2) and the number of branches per plant (Table 3), Which led to an increase in the number of leaves of the plant, and thus increase in the leaf area. This result is in agreement with what was reported by [19, 20] in their study on the faba bean crop.

The results of the same table also indicated that foliar feeding with melatonin had a significant effect on leaf area. The high concentration of melatonin 15 mg.l⁻¹ recorded the highest rate 3840 cm², which did not differ significantly from the concentration of 10 mg. l⁻¹, and gave a mean of 3739 cm². However, the comparison treatment recorded the lower mean of leaf area 3586 cm². The increase in leaf area by increasing the level of leaf nutrition may be due to the important and influential role of melatonin in increasing cell division, expansion and increase in their number, which was positively reflected in the increase in leaf area [21].

The overlap between American genotype and a concentration 15 mg.l⁻¹ recorded the highest average of leaf area, 4808 cm².

Table 4. The role of melatonin and genotypes and overlap in Leaf area (cm²)

| Genotypes | Concentration of melatonin mg.l⁻¹ | Mean |
|-----------|----------------------------------|------|
|           | 0                      | 5    | 10  | 15  |      |
| American  | 4775                  | 4692 | 4802| 4808| 4770 |
| Dutch     | 3175                  | 3133 | 3935| 4128| 3593 |
| Italian   | 3217                  | 3297 | 3205| 3255| 3243 |
| Turkish   | 3177                  | 3325 | 3016| 3168| 3172 |
| Mean      | 3586                  | 3612 | 3739| 3840|      |

L.S.D. 0.05  G = 105.7  M = 151.9  G X M = 222.1

3.5. Dry weight of the plant (g)
Table (5) shows that the American genotype achieved the highest average dry weight of the plant over the other genotypes under study, it Record the highest mean 184.71 g. Whereas the Turkish genotype achieved the lower mean for dry weight of the plant, 157.65 g. This may be due to the fact that the American genotype superiority in most of the vegetative growth traits such as plant height (Table 2),
branches number in plant (Table 3), and leaf area (Table 4), which was positively reflected in the increase this trait. This is agreement with the findings of [4, 22].

The results of the same table show that the dry weight of the plant increased with an increase in melatonin concentrations, until it reached the highest level at 15 mg.l⁻¹, which record a mean of 177.63 g. Then comes the two concentrations 10 and 5 mg. l⁻¹, which recorded an average 173.36 and 169.51 g, respectively. Whereas the comparison treatment recorded the lower mean for dry weight of the plant, 164.30 g. The reason for this may be attributed to the concentration 15 mg.l⁻¹ of melatonin superiority in leaf area in plant (Table 4), which led to an increase the photosynthesis process and an increase in the transfer of processed nutrients to plant, which led to an increase this trait [15].

The effect of overlap between genotypes and melatonin concentrations significantly on the dry weight of the plant. The overlap between the American genotype and the concentration of 15 mg.l⁻¹ gave the highest mean for this trait, 190.57 g. While the overlap between the Turkish genotype and the comparison treatment gave the lower mean dry weight of plant, 147.98 g.

Table 5. The role of melatonin and genotypes and overlap in dry weight of plant (g)

| Genotypes | Concentration of melatonin mg.l⁻¹ | Mean |
|-----------|----------------------------------|------|
|           | 0      | 5      | 10     | 15     |      |
| American  | 180.18 | 182.54 | 185.55 | 190.57 | 184.71 |
| Dutch     | 171.07 | 174.83 | 175.25 | 180.85 | 175.50 |
| Italian   | 157.97 | 164.48 | 171.45 | 173.83 | 166.93 |
| Turkish   | 147.98 | 156.18 | 161.18 | 165.27 | 157.65 |
| Mean      | 164.30 | 169.51 | 173.36 | 177.63 |      |

L.S.D.0.05 G = 1.770 M = 3.887 G X M = 4.575

3.6. Leaf content of chlorophyll (Spad)
The results in Table (6) show the American genotype was superior in giving the highest mean for content of chlorophyll in leaves 48.21 Spad, superior to the rest of the other genotypes (Dutch, Italian and Turkish) with an increase of 6.52%, 36.03% and 5.26%, respectively. The reason for this may be due to the different genotypes in their genetic nature. Or maybe the composition content of chlorophyll is affected as a result of genetic factors interaction with environmental factors and their impact on vegetative growth traits, where the American genotype superiority in plant height, branches number in plant and leaf area (Tables 2, 3 , 4), which led to an increase in the chlorophyll content of the leaves [23 , 24]. This result agreement with results of [25, 26], which indicated that there was a significant difference between the genotypes of the faba bean in this trait.

The results in same table show a significant effect of spraying with melatonin on chlorophyll content. The concentration 15 mg.l⁻¹ superiority in giving the highest mean reached 44.02 spad, and it did not differ significantly from the concentrations 5 and 10 mg. L⁻¹ which record average 43.48 and 43.99 Spad respectively. whereas the comparison treatment gave the lower mean for leaves content of chlorophyll amounted to 43.22 spad. The high concentration of 15 mg.L⁻¹ in the content of chlorophyll in leaves may be attributed to the effective role of melatonin in protecting chloroplasts and delaying leaf aging, which reduces the destruction of chlorophyll, which was positively reflected in the increase in its content in leaves [9].

The overlap between American genotype and the concentration 15 mg.l⁻¹ of melatonin gave the highest mean of chlorophyll content in leaves, which reached 49.57 Spad
Table 6. The role of melatonin and genotypes and overlap in leaf content of chlorophyll (Spad)

| Genotypes | Concentration of melatonin mg.l$^{-1}$ | Mean |
|-----------|----------------------------------------|------|
|           | (G) | 0 | 5 | 10 | 15 |      |
| American  |     | 47.18 | 48.40 | 47.69 | 49.57 | 48.21 |
| Dutch     |     | 44.43 | 45.39 | 45.67 | 45.55 | 45.26 |
| Italian   |     | 35.78 | 34.79 | 36.65 | 34.55 | 35.44 |
| Turkish   |     | 45.49 | 45.33 | 45.95 | 46.41 | 45.80 |
| Mean      |     | 43.22 | 43.48 | 43.99 | 44.02 |      |

L.S.D.0.05 $G = 0.691$ $M = 0.654$ $G \times M = 1.304$

3.7. Pod length (cm)

The results in table (7) show the superiority of American genotype over the other genotypes under study in length of pod, which gave the highest mean, amounting 19.63 cm. Whereas the Italian genotype record the lowest mean of pod length 14.99 cm. The reason may be due to the different genotypes in their genetic nature, as well as their difference in vegetative growth traits, including the length of the pod. This result is in agreement with [27, 28].

Results of the same table also show a significant effect of spraying with melatonin on the length of the pod. The concentration of 15 mg.l$^{-1}$ showed a highest mean, which was 18.15 cm, and it did not differ significantly from the concentration of 10 mg.l$^{-1}$, which recorded the mean 17.69 cm whereas comparison treatment record the lower mean of the pod length, 16.83 cm. The superiority of the concentration 15 mg.l$^{-1}$ in leaf area (Table 4) led to an increase in the process of photosynthesis and the transfer the products of the metabolism to the parts of the plant, including the pod, which led to an increase in its length.

While there was no significant effect of the overlap between the two study factors on the length of the pod.

Table 7. The role of melatonin and genotypes and overlap in Pod length (cm)

| Genotypes | Concentration of melatonin mg.l$^{-1}$ | Mean |
|-----------|----------------------------------------|------|
|           | (G) | 0 | 5 | 10 | 15 |      |
| American  |     | 19.77 | 19.33 | 19.70 | 19.73 | 19.63 |
| Dutch     |     | 16.77 | 16.37 | 18.27 | 18.40 | 17.45 |
| Italian   |     | 13.50 | 15.13 | 1520 | 16.13 | 14.99 |
| Turkish   |     | 17.30 | 17.97 | 17.23 | 18.33 | 17.71 |
| Mean      |     | 16.83 | 17.20 | 17.69 | 18.15 |      |

L.S.D.0.05 $G = 0.830$ $M = 0.693$ $G \times M = ns$

4. Conclusions

The American genotype was distinguished, within the conditions of the experiment, in giving the highest values for most vegetative growth traits compared to the other Dutch, Italian and Turkish genotypes. The vital and effective role of the melatonin growth regulator in increasing the photosynthesis process was reflected positively in increasing the growth traits of the faba bean crop, especially the concentration of 15 mg.L$^{-1}$.

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