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Physiological, Anatomical, Chemical, and Genetic Responses of Two Snap Bean (*Phaseolus vulgaris* L.) Varieties to Invitro Optical Bio-stimulation Induction

Ahmed S. A. Shehata[1], Samah N. Azoz[2], Khaled A. M. Khaled[3], M. Hassan[4], Ghada A. Tawfic[1] and Mahmoud A. M. Fahmy[5] *

[1]Department of Vegetable Crops, Faculty of Agriculture, Cairo University, PO box 12613, Egypt.
[2]Department of Botany, Faculty of Agriculture, Cairo University, PO box 12613 Giza, Egypt.
[3]Department of Genetics, Faculty of Agriculture, Beni-Suef University, PO box 62517, Egypt.
[4]National Institute of Laser Enhanced Science, NILES, Cairo University, PO box 12613, Egypt.
[5]Department of Horticulture, Faculty of Agriculture, Beni-Suef University, PO box 62517, Egypt.

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Corresponding author:
Mahmoud Fahmy

Email:
mahmoud.abdelhamed@agr.bsu.edu.eg

ABSTRACT

This experiment was carried out at Cairo University's Faculty of Agriculture in Giza, Egypt during the 2020 and 2021 seasons in the same location to determine the influence of laser irradiation on the growth, production, chemical content, anatomical structure, and gene expression of dry beans. Two commercial cultivars were used (Tema and Nebraska). The treatments were 5milliwatt power for 30seconds, 5 mill watt power for 120seconds, 20milliwatt power for 30seconds, 20 mill watt power for 120seconds, and control. The result showed that the Tema cultivar combined with 20 mW× 120s enhanced vegetative growth parameters and plant production compared to untreated. The same attributes, chlorophyll a, b, carotenoids, total sugar, total phenol, and antioxidants were significantly affected with the Tema cultivar and 20 mW×120s. Moreover, the highest values of anatomical characters were recorded by treated plants with laser at (20 mW× 120 s) for midvein thickness, Lamina thickness, dimensions of midvein bundle, the number of rows of xylem as well as the mean diameter of the vessel for both cultivars under study. SCoT-cDNA technique identified fragments ranging from 94 to 1609 bp and produced 23 polymorphic bands (52.91% average of polymorphism) with 13 unique bandsmostlyfrom5 milli watt power for 30s. The maximum value of GTS was %40 % at 20-milliwatt power for 120min, while the minimum value was recorded at 22.5 %at5-milliwatt power for 30s and the control. The results revealed the accuracy and benefits of the SCoT-cDNA technique for determining the changes in gene expression in plants exposed to laser radiation.

KEYWORDS: *Phaseolus vulgaris* L, laser irradiation, chemical, genetic attributes, anatomical characters.

1. INTRODUCTION

Beans, a member of the Fabaceae family, are a staple food for individuals of all socioeconomic levels since they provide dietary protein, vitamins, fiber, and complex carbs (Gonzalez et al. 2011).It is a particularly significant and affordable source of proteins, carbohydrates, dietary fibre, vitamins, minerals, and advantageous phytochemicals, antioxidants, and flavonoids (Brigide et al.

2014; Vaz Patto et al. 2015; Mukankusi et al. 2019). After soybean (*Glycine max* L.) and peanut (*Arachis hypogea* L.), snap beans are the third most produced crop in the world, with 26 million tones produced in 2017 (FAO 2017).

Among grain legumes, the common bean has the lowest levels of SNF (Symbiotic Nitrogen Fixation), with an average NDFA (nitrogen from the environment) nitrogen content of 40% (Herridge et al. 2008). The energy supply to the nodules in the snap bean can limit nitrogen fixation (Graham et al. 2003).

One of the more recent agricultural technologies, lasers can be used to physically prime seeds before planting to increase germination and vegetative growth. Lasers also have a variety of additional effects on plants (Nadimi et al. 2021). The Physical method introduced in this study is laser irradiation for seed bio stimulation which is based on photoreceptors activation by light in the visible region (Hernández et al. 2010). Seeds were irradiated via laser, LED, or any other light source to absorb and store radiant energy, the absorbed light energy transformed into chemical energy which affects germination and other growth parameters of plants.

Several researchers studied bio stimulation on crops such as wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and bean (*Phaseolus vulgaris* L.), by using a combined treatment, laser exposure at 532 nm wavelength, and clay-lignocellulose solution. The recorded results illustrate that the combination of treatments significantly improved the germination rate and decrease the germination time (Moșneaga et al. 2018). This work recommended that laser technology could serve as an eco-friendly and safe technique to reduce germination time and enhance plant growth.

Similar work was done on wheat, (*Triticum aestivum* L) seeds that were irradiated using IR laser radiation at □= 980 nm. Studying germination and seedling growth in a lab setting revealed that seed irradiation improved germination and seedling growth. The germination percentage of stem and root growth of the seedlings were also affected by treatment (Michtchenko and Hernández 2010).

Laser treatment of bean (*Phaseolus vulgaris*) seeds has been investigated (Aladjadjiyan 2007). The irradiated regime was continuously by He-Ne laser, intensity 176 Wm^{-2}. Positive results were obtained after laser treatment seeds represented in germination.

Several Studied have been reported on low-power 632.8nm (He-Ne) and 488nm (Ar+) laser on *Vigna radiata* L. (Janayon and Guerrero 2019). The treatment improved mung bean seedling growth, which was obvious in hypocotyl, root length, and seedling mass values.

Samiya et al. (2020) also worked on wheat and investigated the effects of both red and green diode laser irradiation on seed germination, growth, and biochemical parameters. In addition to having a substantial impact on protein concentrations and growth indices including the number of shoots, percentage of germination, and fresh and dry weight of the shoot, irradiation also boosted the activity of the enzymes superoxide dismutase, peroxidase, and catalase.

The wavelength of red He-Ne is 632.8 nm with 2W × m^{2}power density of surface radiation, and an output radiation power of 18mW was used by Możdżeń et al. (2020)to irradiate triticale grains. Germination indexes and some morphological and physiological parameters of seedlings were enhanced. In comparison to the control, fresh and dry weight did not significantly increase. The same was found in chlorophyll content. According to Swathy et al. (2021), all of the brinjal germination indexes, germination times, and seed vigor indexes have been improved. Solanum melongena seedlings were bombarded with a low-level laser in comparison to control groups. Furthermore, it was determined that low-level Laser irradiation with He-Ne improves germination and growth, as evidenced by a significant enhancement of phytohormones and photosynthetic rate, phytochrome and metabolite machinery.

He – Ne laser also was studied on the Flax plant *Linum usitatissimum* L. As a pre-sowing seed priming method, three laser strengths of 40, 60, and 80 mW/m2 are set for 2, 4, and 6 cycles per minute. Alukedi et al. (2021) showed that the majority of the development features and yield components were positively and significantly correlated with laser.

All previous research with such variation in laser irradiation parameters and conditions with enormous changes in plant response starting from seeds germination parameters, seedlings growth morphological, and chemical characters, indicated that using optical bio stimulation via laser or LED should be carefully optimized to maximize positive attributes in the studied plants. To date laser radiation remains poorly understood, few reports have verified the effects of laser treatments on crops, especially vegetable crops.

In the current investigation, we studied the effect of the red He-Ne laser diode at 635 nm with different matrices of exposure powers in mill watt (mW) and irradiation times in seconds (s) on snap beans to study the bio stimulation of seeds germination efficiency, various physiological, and chemical characteristics, and genetic variation as well.

2. MATERIALS AND METHODS

2.1. SITE DESCRIPTION

This experiment was carried out at Faculty of Agriculture, Cairo University, Giza governorate during the 2020 and 2021 seasons in the same location. Seeds of two bean (*Phaseolus vulgaris* L.)cultivars (Nebraska and Tema cultivars) were obtained from the Agriculture Research Center(ARC). Dry bean seeds were hand-sown after irradiation on 1st September 2020 and 8thSeptember 2021 at the greenhouse with three replicates in a split-plot design, where the cultivars were the main plot and the treatments were in the subplot. Each plot consisted of 1 bed 1 m wide and 3 m long. For the subsequent seasons, the necessary precautions against the sickness and insects were taken.

2.2. TREATMENTS

The collected seeds were pre-illuminated with a 630 nm He-Ne laser (equipment whitening, laser II, DMC Equipment Ltd.).

Seeds were divided into nine groups (35 seeds per group from the Nebraska and Tema cultivars): a control group with no irradiation and four laser treatments: (1) 5 mill watt (mW) for 30 seconds; (2) 5 mW for 120 seconds; (3) 20 mW for 30 seconds; and (4) 20 mW for 120 seconds. Lasers were used to individually irradiate the seeds. In order to ensure the best irradiation coverage, the laser source was 5 cm away from the seeds, and the laser head was perpendicular to the samples.

2.3. PLANT GROWTH AND YIELD DETERMINATION

Ten snap bean plants were randomly chosen from each treatment during the vegetative growth stage (70 days after sowing) and measured for plant height, number of leaves, number of branches, leaf area (cm2), chlorophyll (SPAD), fresh plant weight (g), and dry plant weight (g).

In each plot over the two experimental years, ten plants were chosen and marked in order to measure yield components like seed yield (Kg/fed), seed yield per plant, and the number of pods per plant. By accounting for the number of pods, seeds, and seed weights on these chosen plants during harvest, the yield components for each treatment were determined.

2.4. CHEMICAL CHARACTERISTICS

Bean leaves' levels of chlorophyll a, b, and carotenoid were measured using a spectrophotometer. In brief, 1 g It was extracted from the sample in 10 mL of N, N-dimethylformamide for 48 hours. According to Moran (1982), filtration paper was purified the extract and measured at 663, 647 and 470nm, with the results expressed in mg/g (FW). The phenol sulfuric acid method for determining total sugar content was described by Dubois et al. (1956).The colour extract was measured at 490 nm using a spectrophotometer (UNICO S2100, Cole Parmer Instruments, Vernon Hills, IL, USA). With the aid of a glucose standard curve, the results were translated into mg/100 g of fresh weight. Total phenolic compounds were determined using the Folin-Ciocalteu method, which is based on the colorimetric reaction of phenols, with some slight changes (Slinkard and Singleton 1977).At 765 nm, absorbance was measured. TPC was measured in mg/100 g fresh fruit as gallic acid equivalents (GAE).

According to Sanchez-Moreno (2002), the antioxidant activity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Corporation, St. Louis, MO, USA) was assessed using its scavenging activity against free radicals.

Equation was used to compute the antioxidant activity as a percentage of inhibition:

$$\text{Inhibition (\%)} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 10$$

where sample and control are the corresponding absorbances of the sample and the control.

2.5. ANATOMICAL STUDIES

Tested material included the blade of the terminal leaflet of the fourth compound leaf developed on the third branch of the main stem of *Phaseolus vulgaris* cultivars (Nebraska and Tema) as a control, affected by the He-Ne laser at (5,20 mW) and (30, 120 s), respectively, which were taken after 75 days from sowing date. Almost 1.0 cm of the specimens were killer and fixed in FAA solution (50 mL ethyl alcohol 70%, 35 mL water,10 mL formalin and 5 mL glacial acetic). for at least 48 h. The chosen materials were washed in 30% ethyl alcohol, dehydrated in a normal butyl and ethanol alcohol series, embedded in paraffin wax with a melting point of 56°C, sectioned to a thickness of 15 µm stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam in accordance with Mohammed and Guma, (2015). Transverse sections were done with a Leica Microtome RM 2125, and then micro graphed and measured using a Leica Light Image Analysis System DM 750 at the Faculty of Agriculture, Cairo University-Research Park (CURP). The following parameters were measured: thickness of the midvien (µm), palisade tissue (µm), lamina (µm) and spongy tissue (µm), bundles dimension (length –width) (µm), number of xylem rows/midvein bundle, number of vascular bundles, and mean vessels diameter (µm).

2.6. MOLECULAR ANALYSIS AND GENE EXPRESSION

2.6.1. RNA ISOLATION AND CDNASYNTHESIS

Total RNA was isolated from leaf samples of the untreated (control) and treated snap beans using TRIzol® reagent (Rio et al. 2010). Then, cDNA synthesis was conducted using the Techni TC-512 PCR system with a Thermo Scientific Kit (RevertAid™ First Strand cDNA Synthesis) as described by (AL-Taweel et al. 2019).

2.6.2. CDNA -SCOTAMPLIFICATION AND DETECTION

The PCR reaction was performed in a total volume of 25 µL using 10 SCoT primers (Table 1) for the study of expression profiling. The cDNA concentration was approximately 40 ng for PCR amplification with SCoT primer (30 pmol). PCR amplification and gel electrophoresis were carried out using an MJ 200CT Thermal Cycler and submarine gel BioRad as described by (AL-Taweel et al. 2019). The banding patterns were photographed using the JSC Gel Documentation system.

Table 1. Primers sequences, Tm, and GC% of the eighteen SCoT used in PCR reactions.

Primer	Primer ID	Primer Sequence 5` to 3`	GC%	Tm(oC)
1	SCOT 18	ACCATGGCTACCACCGCC	67.00	60.7
2	SCOT 19	ACCATGGCTACCACCGGC	67.00	60.7
3	SCOT 20	ACCATGGCTACCACCGCG	67.00	60.7
4	SCOT 21	ACGACATGGCGACCCACA	61.00	58.4
5	SCOT 22	AACCATGGCTACCACCAC	56.00	56.1
6	SCOT 23	CACCATGGCTACCACCAG	61.00	58.4
7	SCOT 24	CACCATGGCTACCACCAT	56.00	56.1
8	SCOT 25	ACCATGGCTACCACCGGG	67.00	60.7
9	SCOT 26	ACCATGGCTACCACCGTC	61.00	58.4
10	SCOT 27	ACCATGGCTACCACCGTG	61.00	58.4

2.6.3. GEL READING AND ANALYSIS OF CDNA-SCOTEXPRESSION BANDING PATTERNS

CLIQS software (TotalLab Ltd) was used to resolve cDNA-SCoT expression banding patterns. Visible cDNA-amplicons (RNA-derived cDNA amplicons) specified by the cDNA-SCoT were scored for each primer and recorded using a present (0) and absent (1) binary data. Adhikari et al. 2015 method was used to perform the cDNA-SCoT profile from this data. Three types of amplicons were observed dependent on color discrimination: EcDNA-amplicons which described treatments enhanced genes, ScDNA-amplicons referred to the silenced genes due to treatments and McDNA-amplicons that referred to control. TheSCoT-primers efficiency was evaluated through calculation of PIC polymorphic information content (Gorji et al. 2011)and Rp the resolving power (Prevost and Wilkinson 1999). CLIQS software resolved the comparing of band intensity of control and the McDNA-amplicons. The equation reported by was used to calculate Genomic template stability % (GTS) (Liu et al. 2007).

2.7. STATISTICAL ANALYSIS

Using the computer application "MSTATC," a randomized complete block design with two factors was used to analyze all of the data obtained from six replicates over two growing seasons (Gomez and Gomez 1984). The LSD test was used to assess changes across treatment modalities, with p 0.05 indicating statistical significance (Snedecor and Cochran, 1980).

3. RESULTS

3.1. EFFECTS OF LASER-TREATMENT ON VEGETATIVE GROWTH PARAMETERS

All vegetative growth characters of the snap beans cultivars used in this investigation were enhanced by the 20 mW × 120 s treatment. (Table 2). Plant height had the percentage of 28.2% and 26.82%, leaf number/plant recorded 31.39% and 31.35%, leaf area measured 34.49% and 34.18%, and the number of branches were 54.29% and 51.08% in both seasons, respectively, in comparison to the control.

Table 2. Effect of laser treatment on vegetative growth parameters at 70 days after sowing in 2020 and 2021.

TREATMENT	plant height (cm)		No. leaves/plant		No.branches		leaf area (cm^2)	
	2020	2021	2020	2021	2020	2021	2020	2021
5 mW × 30 s	50.8	53.09	16.01	16.33	2.88	2.65	229.2	237.4
5 mW × 120 s	52.05	54.05	16.08	16.41	3.30	3.10	247.7	254.6
20 mW × 30 s	51.07	53.22	18.49	18.87	3.56	3.29	258.5	265.2
20 mW × 120 s	53.3	55.22	19.67	20.07	3.95	3.49	282.3	288.9
CONTROL	41.57	43.54	14.97	15.28	2.56	2.31	209.9	215.3
LSD 0.05	0.97	0.35	0.98	1.00	0.84	0.57	1.05	0.90

TREATMENT	Chlorophyll (SPAD)		Fresh weight (g)		Dry weight (g)	
	2020	2021	2020	2021	2020	2021
5 mW × 30 s	44.36	46.03	514.9	531.7	71.45	73.83
5 mW × 120 s	45.12	46.73	525.8	541.3	72.57	75.15
20 mW × 30 s	45.85	47.15	505.3	518.2	61.97	63.97
20 mW × 120 s	46.87	48.80	510.2	522.3	72.50	74.56
CONTROL	42.33	44.5	374.1	382.4	50.52	52.57
LSD 0.05	0.76	1.60	1.08	0.82	1.51	1.17

In addition, the fresh weight and dry weight were also both significantly affected by the laser treatments, the largest increase in fresh (40.55% and 41.55%) and dry (43.64% and 42.95%) weight were observed with the 5 mW × 120 s treatment, respectively in both seasons. Furthermore, the 5 mW×30 s and20 mW×120 treatments resulted in the highest dry weight values in the first season relative to untreated. The 20 mW× 120 treatment increased chlorophyll content by 10.72% and 9.6% respectively in both seasons (Table 2).

3.2. INTERACTIONS BETWEEN THE LASER TREATMENT AND SNAP BEAN CULTIVAR AND THE IMPACTS ON VEGETATIVE GROWTH PARAMETERS

The results revealed that there was a specific response for the vegetative growth parameters of each snap bean cultivar to the laser treatments (Figure 1). The20 mW× 120 s for the Tema cultivar significantly increased plant height(31.33% and31.99%),number of leaves(31.03% and 30.22%), leaf area (34.4% and 38.35%), and the number of branches (55.92% and51.02%) in both seasons, respectively (Fig. 1A, 1B, 1D, 1C).

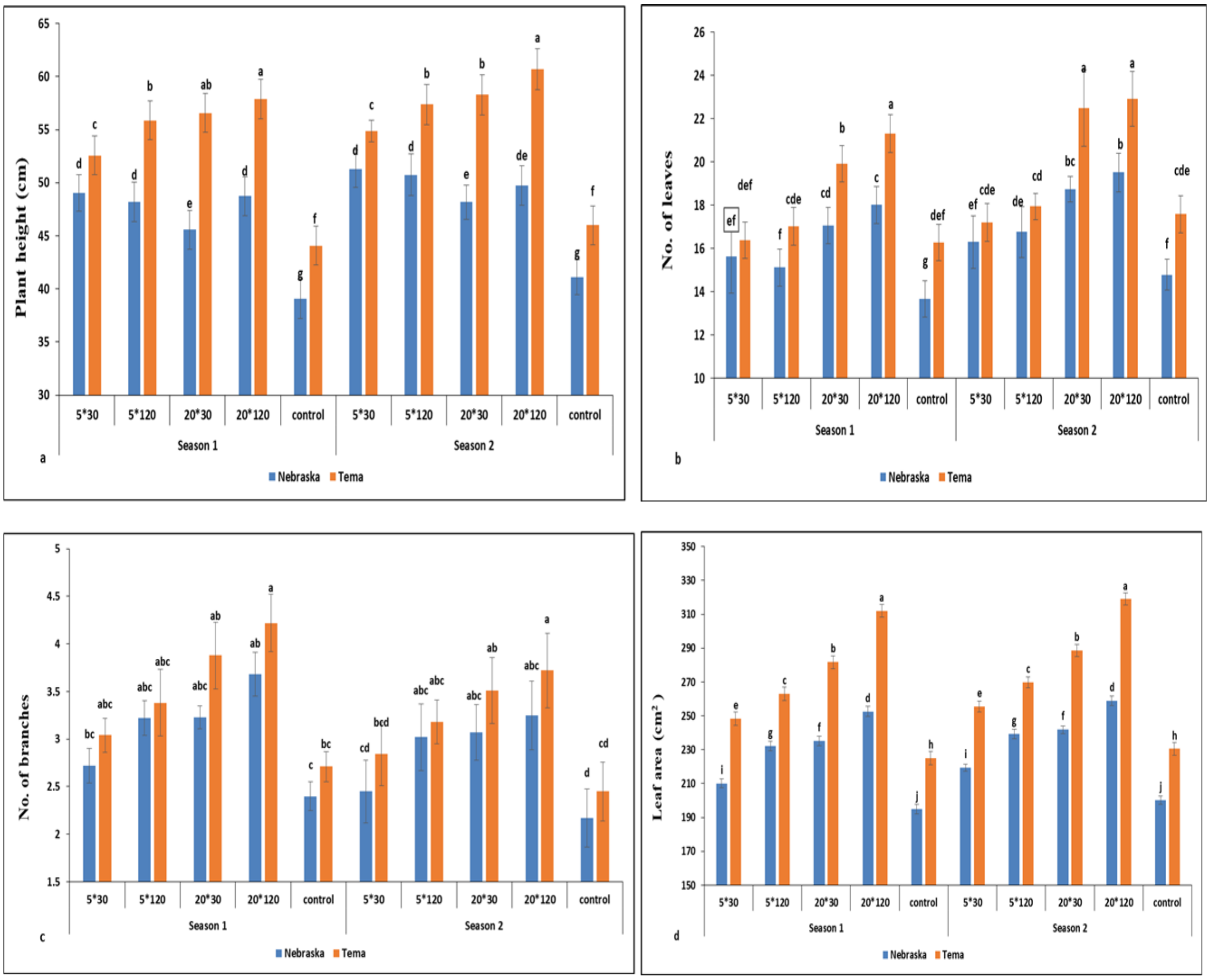


Fig 1. Effects of the interaction between cultivars and laser treatment on vegetative growth parametersat 70 days after sowing in 2020 and 2021. (A) Plant height (cm), (B) No. of leaves, (C) No. of branches, and (D) Leaf area (cm^2).

All laser treatments increased the fresh and dry weight in both cultivars (Figure 2). The highest percentages for the fresh (58.02% and 58.6%), and dry (39.87% and 38.01%)weights were recorded with the 20 mW × 120s treatment for the Tema cultivarin both seasons, respectively (Fig. 2B, 2C),compared to the control. The 5 mW × 30 treatment of the Tema cultivar resulted in the highest dry weight value during the second season.

All treatments considerably enhanced the chlorophyll content, but the Tema cultivars' 20 mW 120 s treatment produced the greatest value, increasing it by 9.4% and 8.4% in both seasons, respectively (Fig. 2A), in comparison to the control.

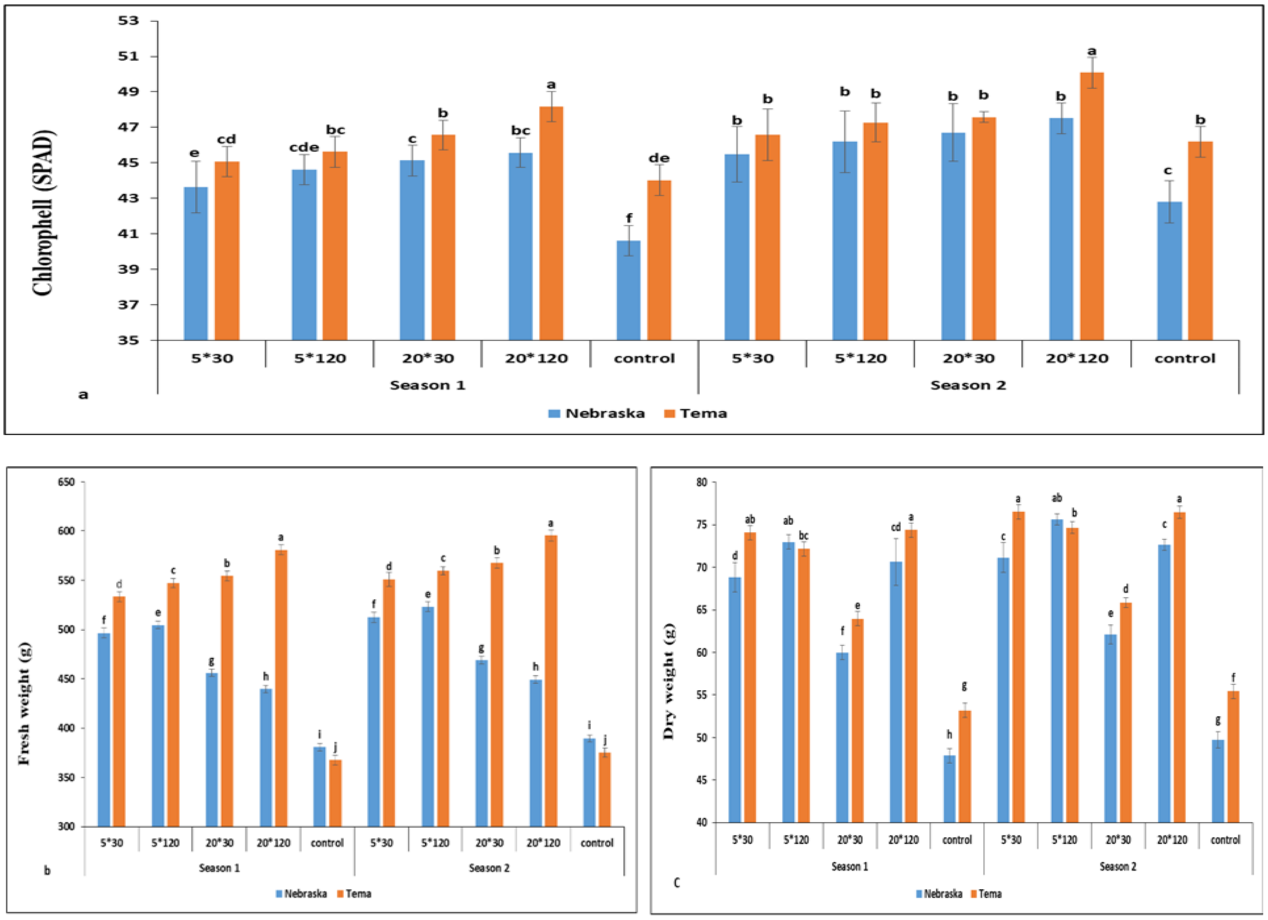


Fig 2. Effects of the interaction between cultivars and laser treatment on vegetative growth parameters at 70 days after sowing in 2020 and 2021. (A) Chlorophyll (SPAD), (B) Fresh weight (g), and (C) Dry weight (g)

3.3. EFFECTS OF LASER TREATMENT ON YIELD AND ITS COMPONENTS

In line with the previous research, the laser treatment significantly enhanced yield and its components such as the number of pods /plant, dry seed /plant, and dry seed /feddan. The 20 mW× 30 s and 20 mW× 120 treatment increased the number of pods by(13% and 13%) and (16.5% and 16.5 %), respectively in both seasons relative to the control (Table 3).

Also, the 20 mW 120 s treatment had the highest dry seed yield/plant and dry seed/fed (Table 3); these yields were considerably boosted by laser treatments, rising by 23.4% for dry seed yield/plant in both seasons and 22.3% for dry seed/fed in both seasons.

Table 3. Effect of laser treatment on yield and its components in 2020 and 2021.

TREATMENT	Seed yield/(kg/fed)		Seed yield/ plant (g)		No.of pods/plant	
	2020	2021	2020	2021	2020	2021
5 mW × 30 s	1994	2040	33.21	33.88	26.22	26.75
5 mW × 120 s	2025	2074	32.89	33.56	25.83	26.35
20 mW × 30 s	2124	2173	34.22	34.91	27.32	27.88
20 mW × 120 s	2275	2329	37.26	38.02	28.17	28.75
CONTROL	1860	1903	30.18	30.80	24.17	24.67
LSD 0.05	1.60	4.98	0.98	1.00	1.01	1.03

3.4. INTERACTION BETWEEN THE LASER TREATMENT AND SNAP BEAN CULTIVARAND THE IMPACTS ON YIELD AND ITS COMPONENTS

The number of pods, seed yield/plant, and seed yield/fed all significantly increased for the Tema cultivars with the 20 mW × 120 treatment, and the highest number of pods increased by 19.09% and19.25%, dry seed yield/ plant increased by 27.46% and 27.5% and dry seed yield/ fed increased by 26.04% and 26.08 in both seasons, in comparison with the control (Figure 3C, 3B, 3A).

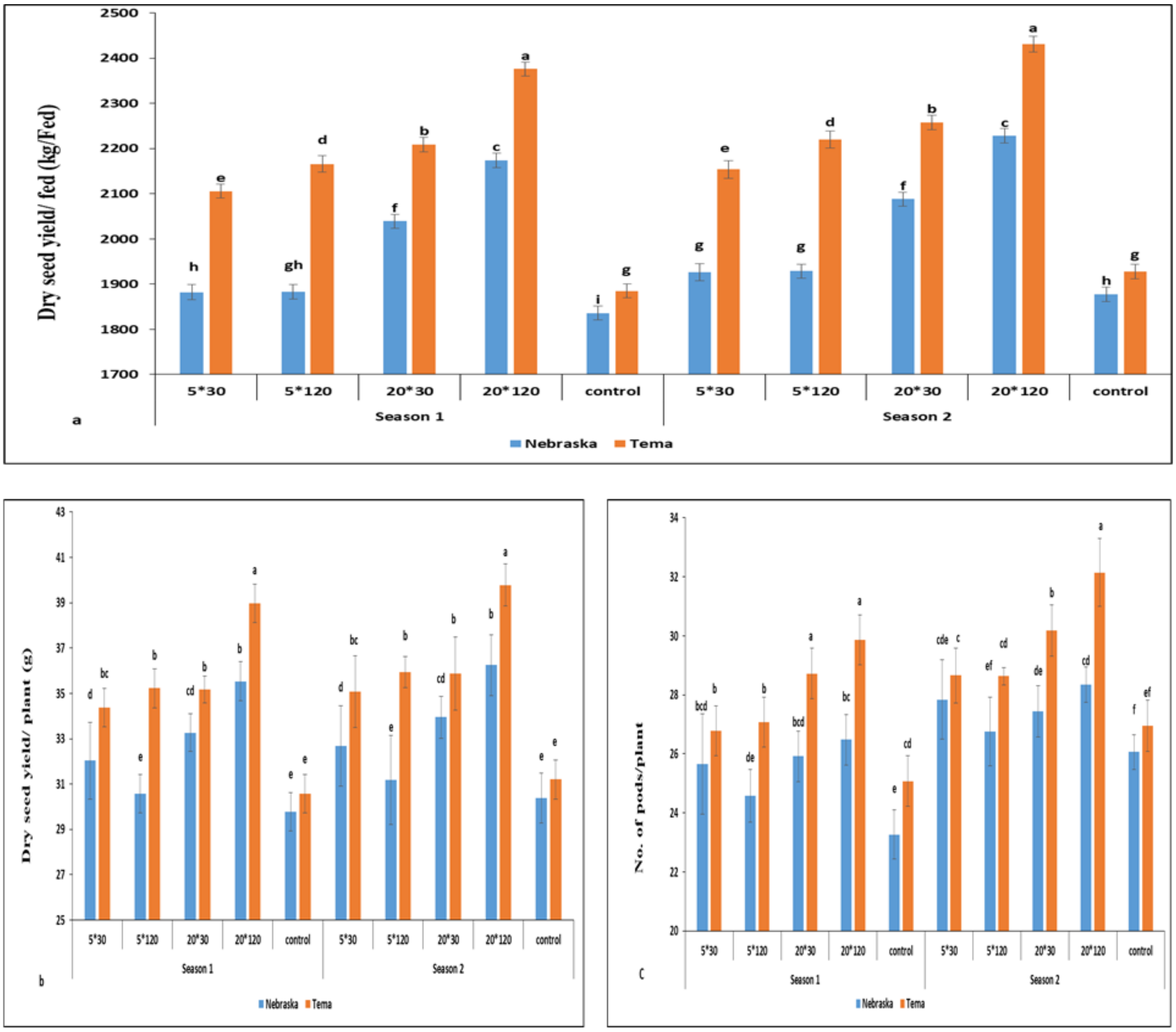


Fig 3. Effect of the interaction between cultivars and laser treatment on vegetative growth parameters at 70 days after sowing in 2020 and 2021. (A) Seed yield/feddan, (B) Seed yield/ plant, and (C) No.of pods/plant.

3.5. EFFECT OF LASER TREATMENT ON CHEMICAL CONTENT

There were substantial variances in the chemical level concentrations between all laser applications in both seasons (Table4).The seeds exposed to the 20 mW × 120 s treatment had increased carotenoid concentrations (64.615% and 67.14%), total sugars(79.1% and 66.79%),chlorophyll a (69.9 % and 72.65%), chlorophyll b(97.03% and 81.97%), total phenols (31.47% and 29.63%), and antioxidants (60.58% and 63.04%), respectively in both seasons.

Table 4. Effect of laser treatment on chemical content in leaves at70 days after sowing n 2020 and 2021.

TREATMENT	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Carotenoid(mg/g)	
	2020	2021	2020	2021	2020	2021
5 mW × 30 s	4.96	5.34	4.24	4.75	2.22	2.42
5 mW × 120 s	5.84	5.97	5.26	5.52	2.70	2.65
20 mW × 30 s	7.21	7.43	6.20	6.14	2.88	2.88
20 mW × 120 s	7.87	8.26	7.31	7.37	3.21	3.51
CONTROL	4.63	4.79	3.71	4.05	1.95	2.10
LSD 0.05	1.08	0.81	1.03	0.89	0.92	0.90

TREATMENT	Total sugar content (mg/100g)		Total Phenolcontent (mg/100g)		Antioxidant (%)	
	2020	2021	2020	2021	2020	2021
5 mW × 30 s	5.63	5.72	127.3	127.6	24.46	25.32
5 mW × 120 s	6.34	6.46	133.5	133.7	27.42	28.37
20 mW × 30 s	7.45	7.79	138.1	139.7	31.13	32.66
20 mW × 120 s	8.85	8.44	146.6	150.9	32.68	34.55
CONTROL	4.94	5.06	111.5	116.4	20.35	21.19
LSD 0.05	0.56	0.65	4.06	5.02	4.58	3.95

3.6.INTERACTION BETWEEN THE LASER TREATMENT AND SNAP BEAN CULTIVARAND THE EFFECTS ON CHEMICAL COMPOUNDS

When the Tema cultivar was treated with 20 mW× 120s (Figure 4C) it resulted in the highest carotenoid level as it increased by 62.08% and 65.85% in both seasons, respectively in comparison with the control. When the Nebraska cultivar was treated with 20 mW× 120 sit resulted in an increase of 67.17% in the second season compared to the control.

The 20 mW× 120 treatment for the Nebraska and Tema cultivars resulted in the highest level of total sugar content in both seasons, as it increased by 80.27%, 77.9%, and 65.22%, 68.9% respectively (Fig. 4D).

Furthermore, the 20 mW× 30 s treatment for both the Nebraska and Tema cultivars in the second season resulted in increases of 55.5% and 52.78% respectively.

In the present study, chlorophyll a and chlorophyll b were found to significantly increase within the Tema cultivar treated with 20 mW× 120s, chlorophyll a increased by 67.5% and 68.9 % and chlorophyll b increased by 96.9% and76.16% in both seasons, respectively (Fig. 4A, 4B). The20 mW× 30 s treatment for the Nebraska cultivar resulted in the highest percentage of chlorophyll a,asit increased by 72.85% and 75.91% respectively in both seasons. When the Nebraska cultivar was treated with 20 mW× 120 s it resulted in an increased in Chlb of 97% in the first season.

For the Tema cultivar with the 20 mW× 120 s treatment, the total phenol increased by 31.79% and 28.9%, antioxidants increased by 68.23 % and 61.0%, respectively in both seasons (Fig. 4E, 4F). Furthermore, when the Tema cultivar was treated with 20 mW× 30 s it resulted in an increase of 60.9% in the first season for antioxidant content.

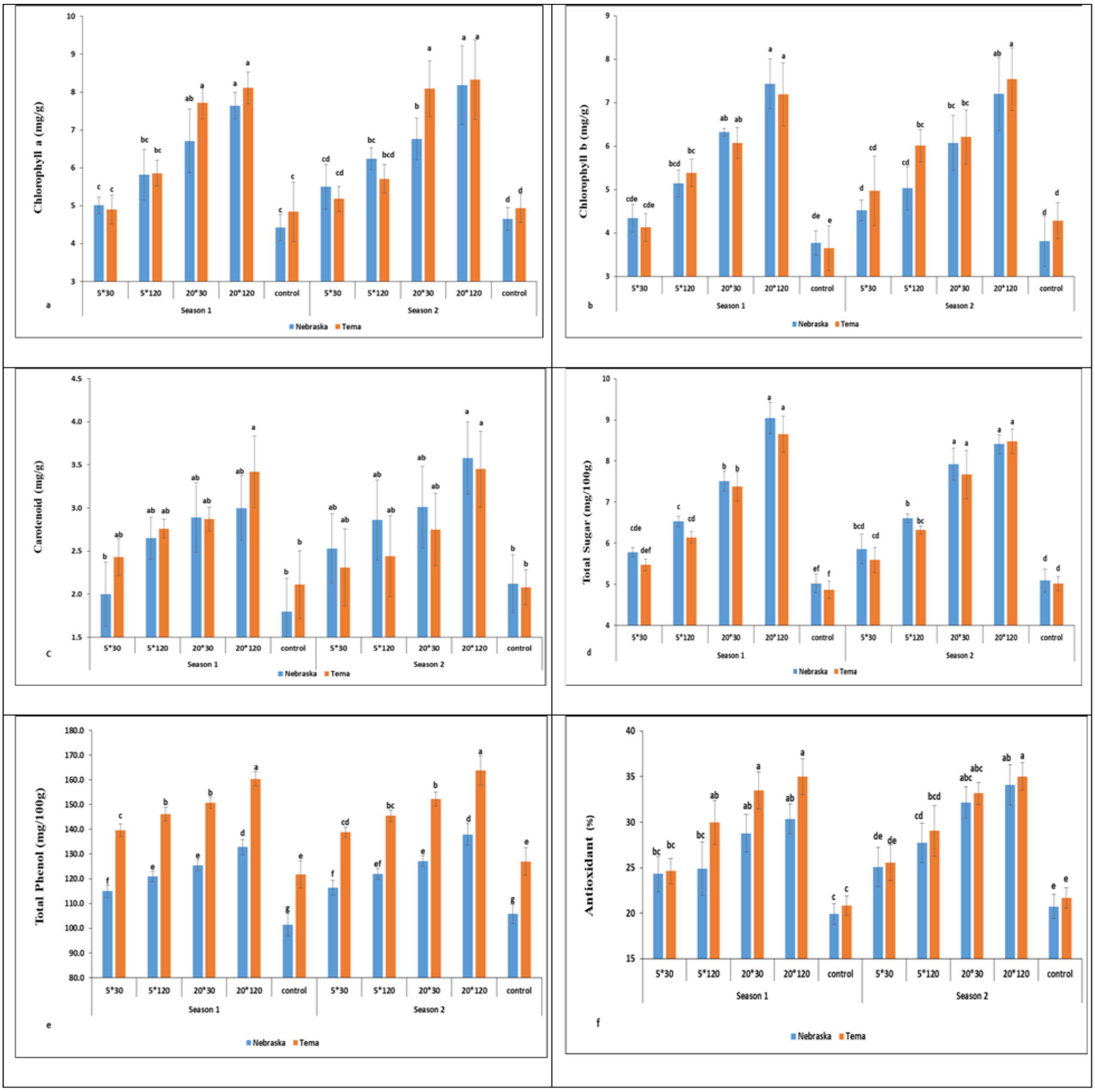


Figure 4. Effect of the interaction between cultivars and laser treatment on vegetative growth parameters at 70 days after sowing in 2020 and 2021. (A) chlorophyll a (mg/g), (B) chlorophyll b (mg/g), (C) Carotenoid (mg/g), (D) Total Sugar Contents (mg/100g), (E) Total phenolic compounds (mg/100g), and (F) Antioxidant activity (%)

3.7. ANATOMICAL STUDIES

3.7.2 The effect of laser treatment on Phaseolus vulgaris anatomical parameters (Nebraska cultivar)

As shown in Table (5) and Figure (5) the treatments with the He-Ne laser at 5 and 20 mW for 30 and 120 s increased all the histological features investigated. The 20 mW×120 s gave the highest increase in the midvein thickness (4.92%) when compared to untreated control. The vascular bundle of the midvein also increased as a result of the increase in length and width, number of xylem rows, and mean diameter of vessels by 6.46%, 11.36%, 8.88% and 1.35%, respectively, when compared with the control. The 5 mW × 30 s treatment resulted in the largest increase in the lamina thickness (24.27%) relative to control. This is due the increase in the thickness of palisade and spongy tissue by 36.36% and 13.75 %, respectively, when compared with the control.

Table 5. Measurements (µm) and counts of some histological aspects in the cross sections through the blade of the terminal leaflet of *Phaseolus vulgaris* (Nebraska cultivar), aged 75 days, exposed to treatments with He-Ne laser(Means of three sections from three specimens)

Histological aspects	Treatments				
	Control	T (5 mW×30 s)	±% to control	T (20 mW×120 s)	±% to control
Midvein thickness	1095.02	1104.53	0.86	1148.95	4.92
Lamina thickness	186.30	231.53	24.27	220.07	18.12
Palisade tissue thickness	83.07	113.28	36.36	97.28	17.10
Spongy tissue thickness	103.91	118.20	13.75	120.96	16.40
Dimension of midvein bundle:					
Depth (Length)	383.85	394.90	2.87	408.65	6.46
Width	336.35	370.52	10.15	374.56	11.36
No. of xylem rows/midvein bundle	4.5	4.7	4.44	4.9	8.88
Mean diameter of vessels	34.64	34.90	0.75	35.11	1.35

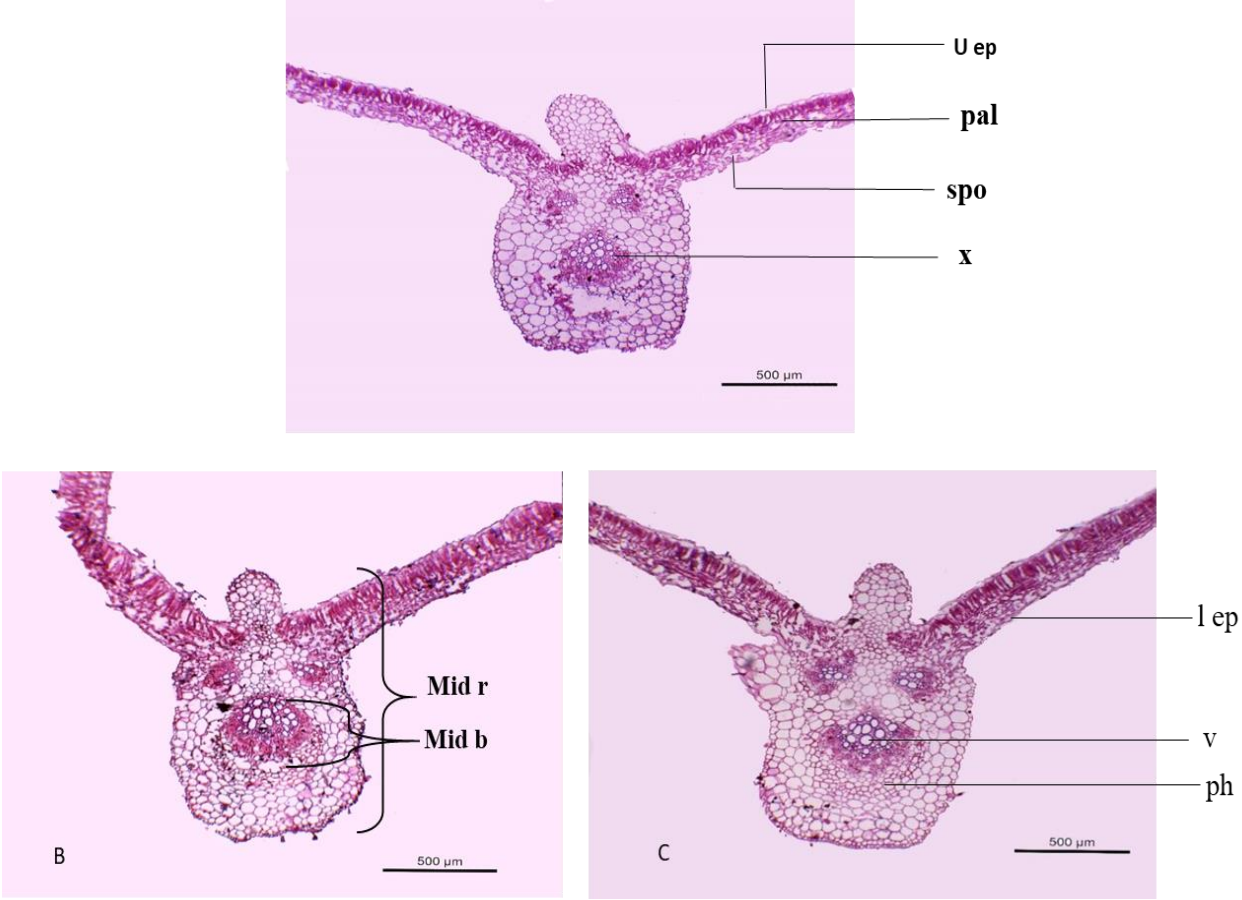


Figure 5. Transverse sections through the blade of the terminal leaflet of *Phaseolus vulgaris* (Nebraska cultivar), aged 75 days, exposed to treatments with He-Ne laser.

Scale bars = 500 µm.

A- From the untreated plants (control) B- From the plants exposed to T(5 mW×30 s) C- From the plants exposed to T(20 mW×120 s)

Details: l ep, lower epidermis; mid b, midvein bundle; mid r, midvein region; spo, spongy tissue; pal, palisade tissue; ph, phloem; x , xylem; v, vessels and u ep, upper epidermis.

3.7.3 The effect of laser treatment on the anatomical parameters of *Phaseolus vulgaris* (Tema cultivar)

The data in Table (6) and Figure (6) show that the *Phaseolus vulgaris* (Tema cultivar) had a poor response to the treatments with He-Ne laser. Nevertheless, both treatments improved the aspects investigated when compared with the control. The 20 mW×120 s resulted in the highest values for the midvein and lamina thickness, as they increased by 2.28% and 2.66 % when compared with the control. This is a result of the increase in the palisade and spongy tissues thickness by 2.80% and 1.84% when compared with the control. The vascular bundle of midvein was also increased in size because the increase in length and width of the bundle, number of xylem rows and mean diameter of the vessels by 3.55%, 13.54%, 6.54% and 0.40%, respectively, when compared with the control.

The stimulative effect of 20 mW×120 s treatment on growth and yield of two cultivars may be due to that 20 mW×120 s treatment increased midvein and lamina thickness, the palisade and spongy tissues thickness, dimensions of midvein bundle, number of xylem rows/ midvein bundle.

Table 6. Measurements (µm) and counts of some histological aspects in the cross sections through the blade of the terminal leaflet of the compound leaf developed on the median portion of the main stem of *Phaseolus vulgaris* (Tema cultivar), aged 75 days, exposed to treatments with He-Ne laser(Means of three sections from three specimens)

Histological aspects	Treatments				
	Control	T (5mW×30 s) ±% to control		T (20mW×120 s) ±% to control	
Midvein thickness	1146.82	1160.14	1.16	1173.00	2.28
Lamina thickness	242.82	247.22	1.81	249.30	2.66
Palisade tissue thickness	129.19	130.32	0.87	132.81	2.80
Spongy tissue thickness	115.19	115.73	0.46	117.31	1.84
Dimensions of midvein bundle:					
Depth (Length)	262.90	269.43	2.48	272.24	3.55
Width	326.46	349.17	6.95	370.69	13.54
No. of xylem rows/midvein bundle	3.1	3.2	3.22	3.3	6.54
Mean diameter of vessel	26.87	26.88	0.03	26.98	0.40

The anatomical structures of two *Phaseolus vulgaris* cultivars (Nebraska and Tema) exposed to the He-Ne laser treatments showed improvement compared with untreated plants. It is evident that the treated plants increased in the midvein thickness, lamina thickness, vessels diameter and xylem rows number. The mesophyll (palisade and spongy tissues) contains chloroplasts, which are responsible for photosynthetic processes. Laser treatments increase the thickness of the mesophyll cells which increase the internal surface area of the leaf and can improve photosynthetic efficiency by increasing the chloroplasts and chlorophyll content, which explains the improvement in all features investigated in this study. Soliman and Harith (2010) found that the anatomy of Acacia farnesiana seed coats treated with an He-Ne red laser (0.03 W/cm2) for 1 min was poorly affected. Bedour et al., (2012) studied the effect of helium neon on gerbera plants and found that the highest number of vascular bundles,

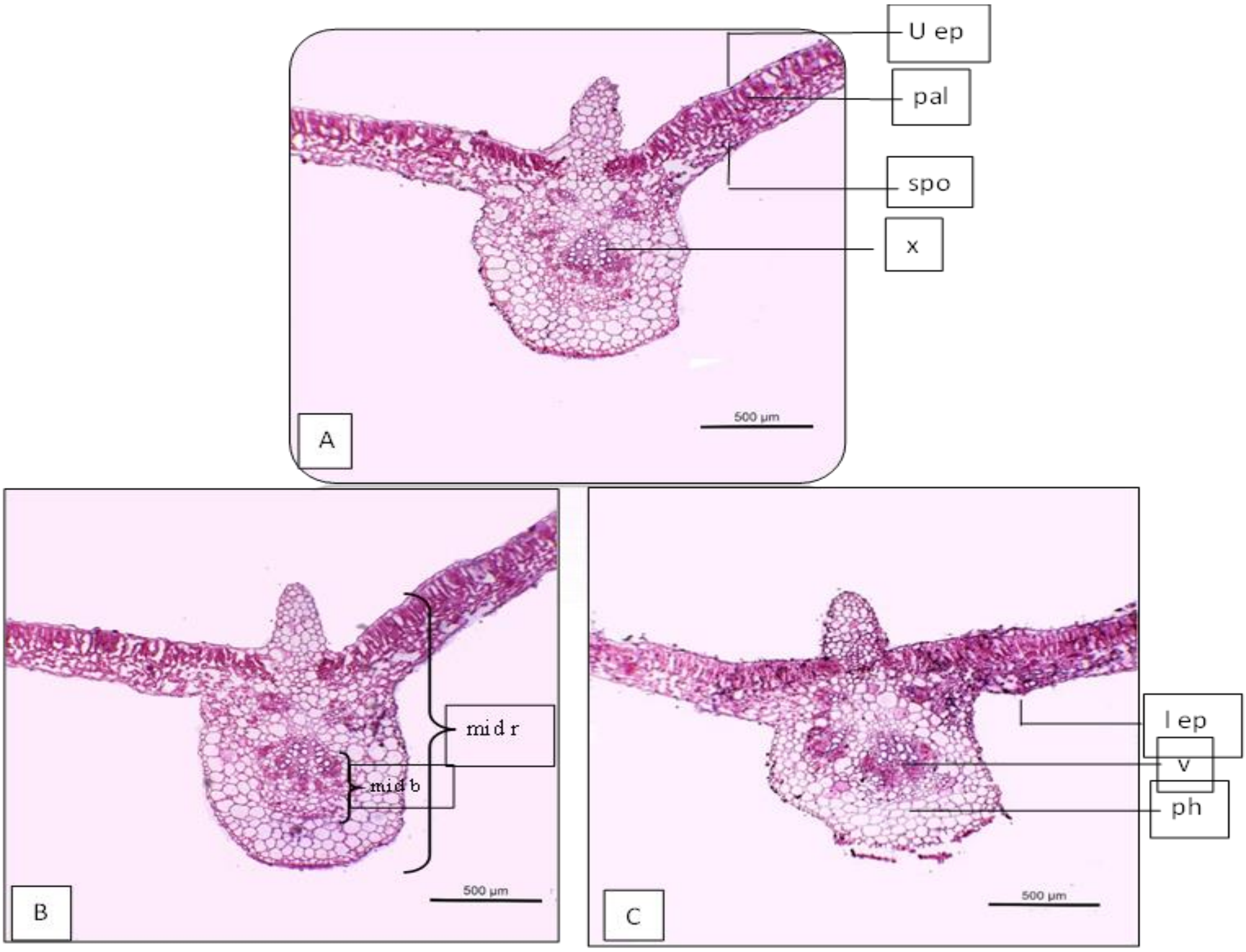


Fig 6. Transverse sections through the blade of the terminal leaflet of *Phaseolus vulgaris* (Tema cultivar), aged 75 days, exposed to treatments with He-Ne laser.

Scale bars = 500 µm.

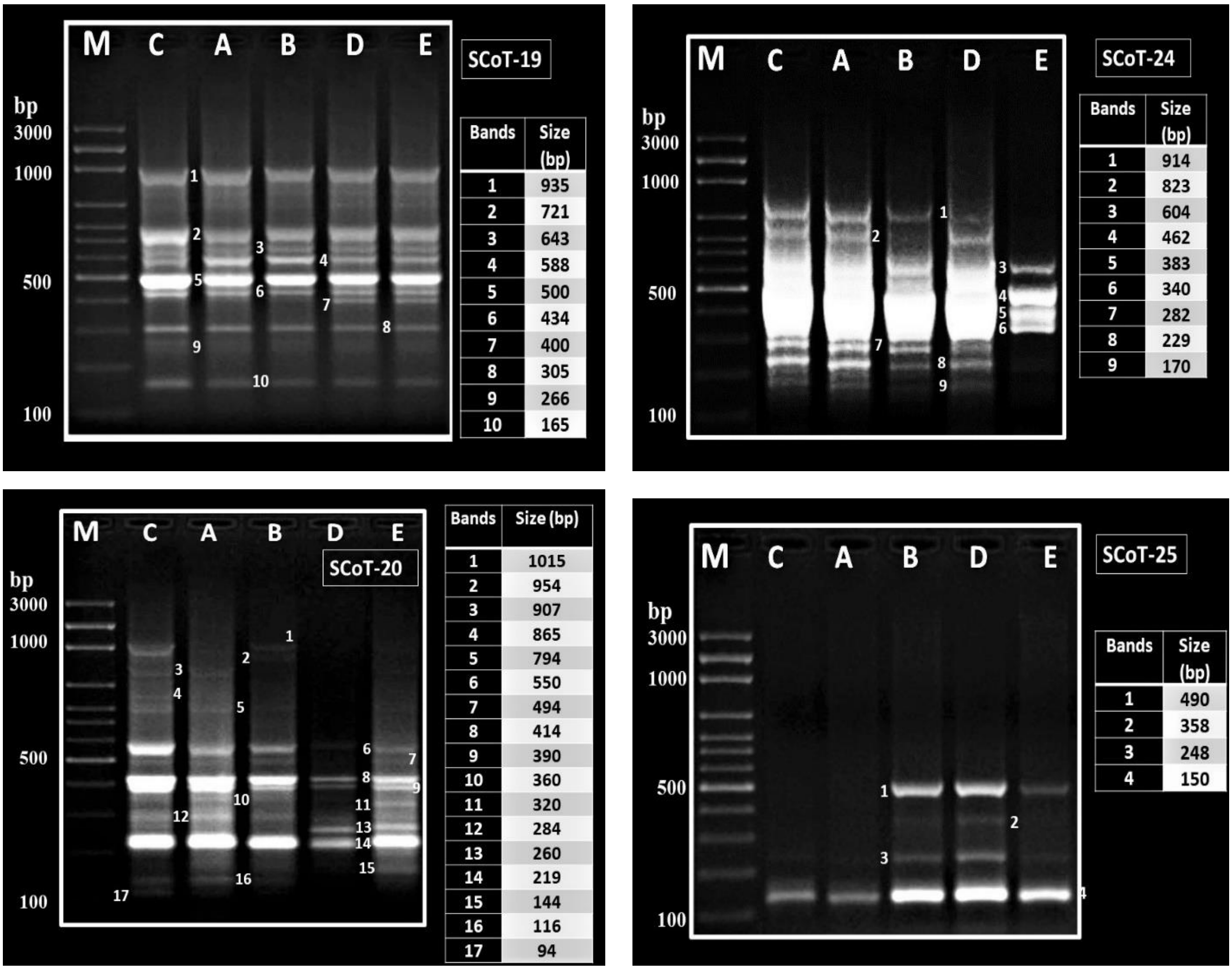
A- From the untreated plants (control) B- From the plants exposed to T(5mW×30 s) C- From the plants exposed to T(20mW×120 s).

Details: l ep, lower epidermis; mid b, midvein bundle; mid r, midvein region; spo, spongy tissue; pal, palisade tissue; ph, phloem; x, xylem; v, vessels, and u ep, upper epidermis

thickness of lamina and number of xylem rows was recorded for plants given a single treatment of helium neon at for 1 min. when compared with the control. The reaction of Celosia argentea var. cristata to various helium neon laser (He-Ne) doses was examined by Metwally et al. in 2013. The findings showed that, in comparison to untreated plants, helium neon laser beams greatly enhanced the number of vascular bundles. The plants treated with (He-Ne) for 3 minutes and 2 minutes showed the greatest values for the number of vascular bundles. Abou-Dahabe et al., (2019) found that when on Eustoma grandiflorium plants were exposed for short time to a helium neon laser and cadmium laser had an increased in midveine thickness, lamina thickness and vascular bundle dimension.

3.8. EFFECT OF LASER IRRADIATION ON THE SNAPBEAN GENOME

Of the 10 primers identified, 4 were capable of forming amplified bands from both the treatment and control samples. A total of 40 amplified bands (loci) were identified, with molecular sizes ranging from 94-1609 bp (Figure.7 and Tables 7 and 8). Of these 40 amplified fragments, 23 produced polymorphic fragments and there were 13 unique bands and they predominantly resulted from the 5mW × 30s treatment. The average percentage of samples polymorphism was 52.91% (Table 7).



SCoT-19

Bands	Size (bp)
1	935
2	721
3	643
4	588
5	500
6	434
7	400
8	305
9	266
10	165

SCoT-24

Bands	Size (bp)
1	914
2	823
3	604
4	462
5	383
6	340
7	282
8	229
9	170

SCoT-20

Bands	Size (bp)
1	1015
2	954
3	907
4	865
5	794
6	550
7	494
8	414
9	390
10	360
11	320
12	284
13	260
14	219
15	144
16	116
17	94

SCoT-25

Bands	Size (bp)
1	490
2	358
3	248
4	150

Fig. 7: Genomic DNA of Snap bean as affected by exposure to He–Ne laser at different time using cDNA-SCoT analysis. M= DNA ladder, C= control, A= 5-milliwatt power for 30 second, B= 5-milliwatt power for 120 second, D= 20-milliwatt power for 30 second and E= 20-milliwatt power for 120 second.

Table 7. Genomic DNA of Snap bean using cDNA-SCoT analysis as affected by different time exposure of He–Ne laser irradiation.

Cod	**Primer**	**Size (bp)**	**Amp bands**	**Monomorphic bands**	**Polymorphic bands**	**Polymorphism percentage**
1	**SCoT-19**	165–935 bp	10	8	2	20
2	**SCoT-20**	94–1015 bp	17	4	13	76.47
3	**SCoT-24**	170–914 bp	9	4	5	55.56
4	**SCoT-25**	206–1609 bp	4	1	3	75
Total no. of bands		–	40	17	23	57.5
Average		–	10	4.25	5.75	–

The highest cDNA-SCoT recorded polymorphic fragments were 31 and 29 at an exposure of 5mW × 30 and 120 s, respectively (Table 7). The GTS % values are shown in Table 8. The maximum GTS was 40 % at 20mW × 120 s followed by 32.5 % at 20mW × 30 s, while the minimum value was recorded at 22.5 %at5mW × 30s and the control.

Table 8. Disappeared and appeared fragments compared to control and GTS% of Snap bean as affected by He–Ne laser using cDNA-SCoT markers

Primer		Treatments					Total
		C	A	B	D	E	
SCoT-19	**935**	+	+	+	+	+	10
	721	+	+	+	+	+	
	643	+	+	+	+	+	
	588	+	+	+	+	+	
	500	+	+	+	+	+	
	434	+	+	+	+	+	
	400	-	-	-	+	+	
	305	+	+	+	+	+	
	266	+	+	-	-	-	
	165	+	+	+	+	+	
SCoT-20	**1015**	+	-	+	-	-	17
	954	-	-	+	-	-	
	907	+	+	-	-	-	
	865	+	+	-	-	-	
	794	+	+	+	-	-	
	550	+	+	+	+	+	
	494	+	+	+	-	+	
	414	+	+	+	+	+	
	390	+	+	+	+	+	
	360	-	+	-	-	-	
	320	-	-	-	-	+	
	284	+	+	+	-	-	
	260	-	-	-	+	+	
	219	+	+	+	+	+	
	144	-	-	-	-	+	
	116	+	+	+	-	-	
	94	+	+	-	-	-	
SCoT-24	**914**	+	+	+	+	-	9
	823	+	+	-	+	-	
	604	+	+	+	+	+	
	462	+	+	+	+	+	
	383	+	+	+	+	+	
	340	+	+	+	+	+	
	282	+	+	+	+	-	
	229	+	+	+	+	-	
	170	+	+	-	+	-	
SCoT-25	**490**	-	-	+	+	+	4
	358	-	-	+	+	-	
	248	-	-	+	+	+	
	150	+	+	+	+	+	
Total number of Bands		31	31	29	27	24	40
GTS%		22.5	22.5	27.5	32.5	40	

(+) appearance of new fragments, (−) disappearance of normal fragments

C= control, A= 5-milliwatt power for 30 second, B= 5-milliwatt power for 120 second, D= 20-milliwatt power for 30 second and E= 20-milliwatt power for 120 second

4. DISCUSSION

Lasers are frequently used in agricultural research as bio stimulators, which are essentially described as the physical process that happens when a seed or plant absorbs low-power laser light and transforms it into chemical energy for later use (Gładyszewska 2006; Hasan et al. 2020). The seeds' physiological and metabolic processes may be triggered by the absorbed energy, potentially enhancing their general performance (Hernández et al. 2010). Laser exposure to seeds can dramatically improve growth traits such plant height, number of leaves, leaf area, number of branches, and chlorophyll content.

Previous researches have demonstrated the advantageous impacts of laser treatments on the characteristics of plants (Vasilevski 2003andHoseini et al. 2013). The seeds are exposed to laser energy during laser therapy, and they absorb this energy, which is then converted into chemical energy for use during germination and growth (Chen et al. 2005aand Aladjadjiyan 2012). When subjected to laser light, plants (Hasan et al. 2020)and seedlings (Almuhayawi et al. 2021) were found to have increased fresh weight and nutritional value.

Which can then be utilized to drive plant physiological functions such as germination, photosynthesis, and respiration (Asghar et al. 2016). Besides that, laser irradiation is utilized to increase nitrogen levels, which results in higher protein content, which increases plant organs such as the number of leaves and leaf area, as well as the number of branches (Osman et al. 2009; Noha and El Ghandoor 2011).

Prior studies have discovered that pretreating seeds with lasers before sowing had a positive impact on growth measures (Alvarez et al. 2011, Khalifa and El Ghandoor 2011, Jia and Duan 2013,). The stimulating effect of laser therapy is assumed to be caused through phytochrome-mediated non-photosynthetic light energy transformation. Plant cell membranes such as the plasma lemma, mitochondria, chloroplast, and endoplasmic reticulum contain phytochrome, a component of the photoreceptor system (Sanchez-Hernandez et al. 2015).Both oxidative and photosynthetic phosphorylation are controlled by phytochrome (Michtchenko and Hernández 2010). The endoplasmic reticulum, tonoplast, and plasmalemma all require phytochrome to transmit energy. The light-induced membrane potential is created by this process, which also transforms light energy into transmembrane energy. This transition means that in addition to light-absorbing photoreceptors, a network of interconnected membrane structures within the cell is in charge of transforming and utilizing the light energy that has been absorbed (Hwida et al. 2012).

The results are in line with those of (Chen et al. 2005b), who discovered that Isatis seedlings had a significant increase in biomass and leaf area. Due to the increased leaf area caused by the reflection of laser rays, increased cell division and increased the level of GA and subsequent growth during the vegetative phase (Rybinski and Garczynski 2004; Al-sherbini et al. 2015), as well as improved morphological and physiological features (Możdżeń et al. 2020). Plant height, branch count, and flower count all increased as a result of the laser-mediated elevation of GA3, which was linked to many physiological processes including cell elongation, auxin, and sugar content (Lobna et al. 2014; Rania et al. 2015). Petkova and Cholakov 2002 found that laser irradiation increased phytomass by 7.6% and 5.7% in tomato and cucumber, respectively. Improved photosynthesis may be linked to increased chlorophyll a and total chlorophyll contents in plants that grow under red and blue light for several hours. Higher levels of chlorophyll have been linked to better photosynthesis because chlorophyll is largely responsible for the absorption of light energy required for photosynthesis in green plants (Evans 1988). Cholakov and Petkova 2002 found that laser irradiation resulted in higher quality seedlings. Moreover, Govil et al. (1985) discovered that laser irradiating greengram seeds for 30 minutes caused the largest increase in shoot and root length as well as seedling dry weight. According to Hernández et al.'s (2006) research, laser therapy increased the dry mass of maize seedlings by 63%. According to (Podleony and Podleoena 2004), the laser treatment for faba bean increased plant height, early flowering, and emergence when compared to the control.

According to Chen et al. (2005a), Seed core energy, cotyledon enzyme activity, and cell metabolism rate may all be increased by pretreatment with He-Ne lasers. Due to an increase in cell division cycles (mitosis) brought on by these factors, the length of plant organs during their early development increased. According to (AlSalhi et al. 2018), Wheat seedlings benefit from laser treatment in terms of length and dry weight. The results of this study corroborated those of (Podlesna et al. 2015), who looked at how laser irradiation affected the growth and flowering of white lupine and faba bean. The treated seeds grew bigger more quickly after imbibition than the untreated seeds. Laser-pre-sowing treated seeds from different leguminous species have been shown to increase dry weight, seed emergence rate, blooming time, and time to maturity (Asghar et al. 2016).

It's been widely accepted for a long time that laser light increases plant growth and seed germination. By converting light energy into chemical energy, boosting cell pumping, and raising the electropotential of bio membranes, phytochromes tend to enhance the internal energy of seeds when they absorb a certain wavelength of laser light (Chen et al., 2005b). As a result, the energy generated is employed to promote thermodynamic qualities, quicken germination, and control physiological and biochemical processes. Additionally, this energy quickens the process of cell division and boosts the activity of enzymes like amylase and protease, all of which help plants grow and produce more (Chen et al. 2005a; Asghar et al. 2016; Perveen et al. 2010). The results of this study showed that the quantity of pods/plant, dry seed/plant, and dry seed/feddan greatly increased after laser treatment.

Before planting, He-Ne laser treatment of the seeds encourages emergence, quickens flowering and maturation, and stimulates plant development (Perveen et al. 2011; Qi, et al. 2002).The higher net photosynthetic activity of the laser light is what causes the observed rise in plant growth after laser light therapy (Perveen et al. 2011; Chen et al. 2005b). The output of biomass increases with photosynthetic rate because more sugars and organic acids are produced. Also, it influences the nature of free radicals, the production of hormones, especially indolyl-3-acetic acid, as well as the functions of enzymes like amylase and protease.

Plant nutrition is influenced by secondary metabolites like phenolic compounds (De Beer et al. 2017). According to Tungmunnithum et al. (2018), the presence of phenolic compounds and flavonoids, which are well-known antioxidants, is a clear sign of a plant's nutritional worth. Also, it has been demonstrated that the laser light therapy enhances the development and phenolic content of White lupine and faba bean seedling metabolism (Podle sny et al. 2012). This rise may be due to light's capacity to facilitate photosynthesis and the malonyl-CoA pathway, which is connected to the biosynthesis of phenolic compounds in plants (Kim et al. 2006). Moreover, laser light improved photosynthetic activity, leading to a rise in the synthesis of carbohydrates, which is a precursor for several types of carbon-based secondary metabolites, including polyphenols (Saleh et al. 2018).

As carotenoid production is thought to be influenced by light quality and intensity, carotenoid synthesis has been known to play a significant role in the photosynthetic process.The buildup of carotenoids has been linked to the up-regulation of genes involved in carotenoid production, highlighting the significance of light in this process (Tang et al. 2013).Plants under He-Ne laser irradiation had increased SOD, CAT, and APX enzyme activity, which sped up physiological metabolic processes and improved plant development. Furthermore, appropriate laser irradiation doses increased the concentration of pigment like chlorophyll which enhanced the plant growth (Aladjadjiyan 2002).

Sugars have been linked to development and growth (Wanget al. 2007; Aghalehet al. 2009), hence the current study's enhanced photosynthetic rate could indicate increased sugar generation. Laser light has the potential to influence sprout growth in two ways: either directly by enhancing the thermodynamic and germination factors or indirectly by altering the photosynthetic process. In this context, laser light has been shown to increase chlorophyll content (chlorophyll a and b) in a variety of plants, including *Isatis indogotica*(Chenet al.

2005b), soybean (Asgharet al. 2016), and sunflower (Perveenet al. 2011). Furthermore, laser photon energy is absorbed by chlorophyll, which has a direct impact on photosynthetic intensity (Aladjadjiyan2007),which agrees with our investigation for increasing chlorophyll a and b in the study. Laser light could boost chlorophyll content in this situation (Perveenet al. 2011; Chenet al. 2005b).

The antioxidant activity of edible plant components is regarded as a crucial predictor of their nutritional value (Tang et al. 2013). Additionally, there is some direct data demonstrating a connection between total phenolic and flavonoid content and total antioxidant activity for a number of plant species (Chu et al. 2019).

The anatomical structures of two Phaseolus vulgaris cultivars (Nebraska and Tema) exposed to the He-Ne laser treatments showed improvement compared with untreated plants. It is evident that the treated plants increased in the midvein thickness, lamina thickness, vessels diameter and xylem rows number. The mesophyll (palisade and spongy tissues) contains chloroplasts, which are responsible for photosynthetic processes. Laser treatments increase the thickness of the mesophyll cells which increase the internal surface area of the leaf and can improve photosynthetic efficiency by increasing the chloroplasts and chlorophyll content, which explains the improvement in all features investigated in this study. Soliman and Harith (2010) found that the anatomy of Acacia farnesiana seed coats treated with an He-Ne red laser (0.03 W/cm2) for 1 min was poorly affected. Bedour et al., (2012) studied the effect of helium neon on gerbera plants and found that the highest number of vascular bundles, thickness of lamina and number of xylem rows was recorded for plants given a single treatment of helium neon at for 1 min. when compared with the control. The reaction of Celosia argentea var. cristata to various helium neon laser (He-Ne) doses was examined by Metwally et al. in 2013. The findings showed that, in comparison to untreated plants, helium neon laser beams greatly enhanced the number of vascular bundles. The plants treated with (He-Ne) for 3 minutes and 2 minutes showed the greatest values for the number of vascular bundles. Abou-Dahabe et al., (2019) found that when on Eustoma grandiflorium plants were exposed for short time to a helium neon laser and cadmium laser had an increased in midveine thickness, lamina thickness and vascular bundle dimension.

The cDNA-SCoT sequences revealed significant variations between the The cDNA-SCoT profiles revealed significant variations between normal and He-Ne laser-treated samples using various primers (Figure. 7 and Tables 7 and 8). Ten primers were used to compare the control to samples that had been treated with the He-Ne laser at various intervals. According to Ozturk et al. (2010), variations in DNA profiles indicate nucleotide substitutions or alterations (point mutations) in complicated chromosomal rearrangements. In contrast to the control, the results showed the loss (-) of normal bands and the emergence (+) of new bands. The photoproducts of DNA existence may cause a loss of bands (for example, the dimers of pyrimidine), that decreased amplification of DNA through PCR reactions (Atienzar et al. 2000). Because the probability of acquiring DNA photoproducts increases with the length of the amplified fragment, high molecular weight bands are the most affected by amplified bands disappearing (Atienzar et al. 2000). Mutations (new annealing events) can, however, only cause the formation of new bands when they occur at the same locus. A novel PCR product may require at least 10% mutations (Atienzar et al. 2000). As a result, the additional loci may hint at mutations, whereas loci vanish when DNA is damaged (bands).

DNA replication, DNA form effectiveness, and DNA damage position are all connected to GTS. DNA polymorphisms discovered by the SCoT method in different treatments and in comparison, to the control might be utilized as a discussion tool for future sequencing research and as a helpful method for identifying the inheritable variety among individuals treated with Ray. The modification of the SCoT profile by ray treatments can be interpreted as a change in the stability of the genomic DNA template, and these inheritable goods can be directly compared with variations in other parameters.

The smallest GTS was recorded with 5 mW for 30 seconds and control, and it was 22.5. As the values of GTS and polymorphism are inversely correlated, the samples' exposure to ray radiation for 30 seconds at 5 mW resulted in the lowest GTS and highest polymorphism values. This could be related to increasing agromorphological parameters such as shoot length and weight, root weight, and leaf count.

Because of differences in various growth metrics (root and shoot length) in Snap bean shops, the GTS compared the changes in the cDNA-SCoT biographies. DNA analysis is thought to be a more sensitive test because it can detect transient DNA changes that aren't mutations (Labra etal. 2003).

5. CONCLUSIONS

Based on results of this research, this experiment suggests that Tema plants exposed to 20mW × 120 s irradiation light improved snap bean growth, yield, and biochemical attribute. Moreover, the same enhancement appeared in the anatomical structure of the leaf and gene expression stability. In addition, polymorphism was found in 52.91% of the samples studied. At 5-milliwatt power exposure for 30 and 120 seconds, the maximum cDNA-SCoT recorded polymorphic bands were 31 and 29, respectively. This finding highlights the importance of using laser light in agricultural research and highlights the necessity for additional study to determine the precise mechanisms driving these benefits.

6. ACKNOWLEDGMENTS

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الملخص العربي

الاستجابات الفسيولوجية والتشريحية والكيميائية والجينية لنوعين من الفاصوليا (*Phaseolus vulgaris* L.) للتحفيز الحيوي

أحمد سعيد عبد الله شحاته[١]، سماح نجيب عزوز[٢] ، خالد عدلي محمد خالد[٣]، منار حسن محمد صالح[٤]، غاده أحمد توفيق[١] و محمود عبد الحميد محمد فهمي[٥]

[١]قسم الخضر ، كلية الزراعة ، جامعة القاهرة ، ص.ب ١٢٦١٣ الجيزة ، مصر.
[٢]قسم النبات ، كلية الزراعة ، جامعة القاهرة ، ص.ب ١٢٦١٣ الجيزة ، مصر.
[٣]قسم الوراثة ، كلية الزراعة ، جامعة بني سويف ، ص.ب ٦٢٥١٧ ، مصر.
[٤]المعهد القومي لعلوم الليزر، جامعة القاهرة ، ص.ب ١٢٦١٣ الجيزة ، مصر.
[٥]قسم البساتين ، كلية الزراعة ، جامعة بني سويف ، ص.ب ٦٢٥١٧ ، مصر.

أجريت هذه التجربة في كلية الزراعة بجامعة القاهرة خلال موسمي ٢٠٢٠ و٢٠٢١ في نفس الموقع لتحديد تأثير تشعيع الليزر على النمو والإنتاج والمحتوى الكيميائي والتركيب التشريحي والتعبير الجيني للفاصوليا الجافة. تم استخدام صنفين تجاريين وهما تيما ونبراسكا. وكانت المعاملات عبارة عن طاقة ٥ ملي واط لمدة ٣٠ ثانية، و٥ ميلي واط لمدة ١٢٠ ثانية، و٢٠ ميلي واط لمدة ٣٠ ثانية، و٢٠ ميلي واط لمدة ١٢٠ ثانية، بالإضافة إلى الكنترول. أظهرت النتائج أن صنف تيما مع ٢٠ ميغاواط × ١٢٠ ثانية حسن كل من النمو الخضري والإنتاجية مقارنة بالنباتات الغيرمعاملة. تأثر أيضا كل من الكلوروفيل أ، ب و الكاروتينات و السكريات الكلية و الفينولات الكلية ومضادات الأكسدة تأثرت معنويا مع صنف تيما و ٢٠ ميغاواط × ١٢٠ ثانية. علاوة على ذلك ، تم تسجيل أعلى قيم للصفات التشريحية بالنباتات المعالجة بالليزر عند (٢٠ ميغاواط × ١٢٠ ثانية) لسمك Midvein ، وسمك Lamina ، وأبعاد حزمة midvein ، وعدد صفوف نسيج الخشب وكذلك متوسط قطر الوعاء لكلا الصنفين قيد الدراسة. حددت تقنية SCoT-cDNA شظايا تتراوح من ٩٤ إلى ١٦٠٩ نقطة أساس وأنتجت ٢٣ نطاقًا متعدد الأشكال (٥٢,٩١٪ متوسط تعدد الأشكال) مع ١٣ نطاقًا فريدًا معظمها بقوة ٥ ملي واط لمدة ٣٠ دقيقة. كانت أقصى قيمة لـ GTS٪ 40٪ عند طاقة ٢٠ ملي وات لمدة ١٢٠ ثانية ، بينما تم تسجيل الحد الأدنى للقيمة عند ٢٢,٥٪ عند طاقة ٥ ملي وات لمدة ٣٠ ثانية بالإضافة إلى معاملة الكنترول. كشفت النتائج دقة وفوائد تقنية SCoT-cDNA لتحديد التغيرات في التعبير الجيني في النباتات المعرضة لإشعاع الليزر.

الكلمات المفتاحية: *Phaseolus vulgaris* L، الفاصوليا ، التشعيع بالليزر ، الصفات الكيميائية ، الصفات الوراثية ، الصفات التشريحية.