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Studying the effect of laser stress in combination with mannitol on some morphological parameters of cabbage (*Brassica oleracea* var *L. capitata*) *In vitro*

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Abstract :

The main purpose of this work is to investigate the influence of Nd: YAG (neodymium-doped yttrium aluminum garnet) green laser irradiation on mannitol- induced drought stress in white cabbage (*Brassica oleracea* var *L. capitata*) using *in vitro* technique. Seeds were surface sterilized by using 70% ethanol for 1 min followed by 2% of (NaOCl) for 15 minutes, then irradiated with five doses of green laser at different duration namely (0, 25, 50, 75, 100, and 125 seconds) and then germinated on MS basal medium for 14 days. After seeds germination, the germination percentage, number of shoots. explants^{-1}, shoots height (cm), and number of leaves. plantlets^{-1} was recorded. Results revealed that higher germination 100 % at (0 and 100 and 125 seconds) of laser treatment. Whereas (75 and 100 seconds) were superior in giving highest number of shoots. Explants^{-1}, shoots height and number of leaves. Concerning the effect of laser treatments in combination with mannitol osmotic stress, the results clarified that the highest callus fresh weight was (232.87 mg) at 60 g.l^{-1} of mannitol at 125 seconds of laser treatment, while 40 g.l^{-1} of mannitol in combination with (25, 50 and 125 seconds) recorded the highest callus dry weight (35.11, 33.6 and 33.95 mg) respectively.

Key words: *Brassica oleracea* var *L. capitata,* green laser BA, NAA, seed germination, callus, mannitol.

دراسة تأثير إجهاد الليزر مع المانيتول على بعض المتغيرات المظهرية للملفوف (Brassica oleracea var L. capitata) في المختبر

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مستخلص:

الغرض الرئيسي من هذا العمل هو دراسة تأثير إشعاع الليزر الأخضر Nd: YAG (عقيق الإيتريوم الألومنيوم المشبع بالنيوديميوم) على إجهاد الجفاف الناجم عن المانيتول في الملفوف الأبيض (Brassica oleracea var L. capitata) باستخدام تقنية المختبر. تم تعقيم البذور السطحي باستخدام ٪70 إيثانول لمدة دقيقة واحدة تليها ٪2 من (NaOCl) لمدة 15 دقيقة، ثم تعقيمها بخمس جرعات من الليزر الأخضر بمدد مختلفة وهي (0 ، 25 ، 50 ، 75 ، 100 ، 125 ثانية) ثم نبتت على وسط MS القاعدية لمدة 14 يومًا. بعد إنبات البذور، تم تسجيل نسبة الإنبات، عدد الأفرع. النبيتات 1-، ارتفاع النبتة (سم)، وعدد الأوراق. نباتات 1-. أظهرت النتائج أن أعلى انبات بنسبة ٪100 عند (0 و100 و125 ثانية) من العلاج بالليزر. في حين تفوقت (75 و100 ثانية) في إعطاء أكبر عدد من الأفرع. النبيتات 1-، ارتفاع النبتة وعدد الأوراق. فيما يتعلق بتأثير العلاج بالليزر مع الإجهاد التناضحي للمانيتول، أوضحت النتائج أن أعلى وزن طري للكالس كان (232.87 مجم) عند 60 غم. لتر 1- من المانيتول عند 125 ثانية من العلاج بالليزر، بينما 40 غم. لتر 1- من المانيتول بالاشتراك مع (25 و50 و125 ثانية) سجلت أعلى وزن جاف للكالس (35.11 و33.6 و33.95 مجم) على التوالي.

Introduction:

A laser is described as a beam of light that has distinct optical properties that differ from the wavelength of the light intensity and the properties of the light beam (Gladyszewska, 2011; Sacala *etal*., 2012). It has been discovered in the past century, and has been used in the society from its conception until today. Among their application, its use in agriculture as a biostimulator device. The laser light at low intensity produces bio stimulation when it used on seeds and seedling plants (Chen *et al*., 2005).

In order to get sufficient rates of photosynthesis and chlorophyll concentration, plants require a visible spectrum of light ranging from 400 to 700 nanometers, in which the light that falls within this spectral range affects plant growth differently. Plants use violet and blue light waves, between 400 to 490nm, for vegetative growth. While red light, which falls within narrow spectrum between 580 to 700nm, is used by plants for reproductive growth (Brody Hall, 2023).

From the initial laser application in agriculture until now, different laser light types have been used, from ultraviolet to far infrared, including UV (200-400 nm), visible light (400-700 nm), near infrared radiation (750-2500 nm) and far infrared (5000-10^6 nm). Solid, gaseous and semiconductor lasers have been applied in agriculture, for example: ruby laser (694 nm), He-Ne (632.8 nm), nitrogen laser (337.1 nm), argon (514.5 nm), YAG:Nd laser (532 nm), diode laser (510, 632, 650, 670, 810, 940 and 980 nm), AsAlGa semiconductor laser (650, 660 and 850) and CO2 (10600 nm) (Hernandez *et al*., 2010). Moreover, laser irradiation parameters are important to take into an account at the bio stimulation process such as: wave-length, exposure time, intensity, (D) dosage, irradiation regime number and light type (pulsed or continuous) (Hernandez *et al*., 2010).

Mannitol on the other hand, is a white, solid crystal with the chemical formula $C_6H_8(OH)_6$. It is a sugar alcohol with an osmotic adjustment property that can be used to control osmotic potential in the culture media or nutrient solutions to create water deficit conditions for protein expression or proteomics studies (Chaves and Oliveira, 2004; Zidenga, 2006). Since water scarcity and drought are major factors of agriculture, this chemical is employed in plant cultivation as an inducer of osmotic stress (Chaves and Oliveira, 2004). This substance is used in plant cultivation as an inducer of osmotic stress, since water scarcity and drought problems are among the most important determinants of agriculture. The increase in global warming recently led to a decline in the level of agriculture in general as a result of the exacerbation of drought.

Therefore, an urgent importance emerged to understand the mechanism that the plant adopts to overcome this obstacle. Indeed, plants have a variety of adaptations to cope with various levels of drought stress (Xoconostle-Cazares *et al*., 2010). Drought is the most common abiotic stressor, impacting both plant growth photosynthesis (Flexas *et al*., 2002) as well as modifying plant biochemical properties (Zobayed *et al*., 2007).

The aim of this study is to investigate the influence of Nd:YAG green laser irradiation on improving callus tolerance to water deficiency in the presence of mannitol as an osmotic stressor.

Materials and Methods

Seed sterilization

For the purpose of carrying out this research, mature seeds of the white cabbage plant were obtained from the local market in Baghdad, and surface sterilized using 70% ethanol for 1 minute and then immersed in 2.0% sodium hypochlorite (NaOCl) for 15 minutes with constant stirring, thereafter were washed five times with sterile distilled water to remove any effect of the disinfectants (Hur and Min, 2015).

Culture media preparation

According to the manufacturer's instructions', the Murashige and Skoog, (MS) salts medium was prepared with $7 g.l^{-1}$ of agar and then sterilized by autoclaving at 121 °C for 15 minutes.

Treatment of seeds with laser light

Seeds were exposed to different periods of green laser Nd:YAG (neodymium-doped yttrium aluminum garnet) irradiation of low intensity (532 nm ± 10 wavelength, <100 mW, continues light type) using a laser pointer (Fig,1).

Fig 1: laser pointer (green laser Nd:YAG)

Five irradiation treatments (25 , 50 , 75 , 100 , and 125) seconds in addition to control (without irradiation) were included. Seeds were spread on prepared MS media, and then the culture bottols were incubated in tissue culture room at 25 ± 2 °C with 16 hours of light with 1000 Lux intensity and 8 hours in dark conditions. After 14 days of incubation, germination percentage %, shoot length, No. of shoots. Explants^{-1} and leaves) were recorded.

Callus were initiated from hypocotyls on MS media supplemented with (1 BAP + 1 NAA mg. l^{-1}) (Hasan *et al*., 2021).

Drought stress experiment

A constant weight (100 mg) of callus fresh weight from each treatment was cultured on (MS) media supplemented with mannitol at concentration of (0, 40 and 60 g. l^{-1}). The experiment was performed on three replicates. After 40 days, the fresh and dried weight (mg) of the callus was determined.

Statistical analysis

The experiments were designed in Complete Random Design (CRD), each treatment was performed in triplicates. The collected data was analyzed using Gen Stat program (Glaser and Biggs, 2010) while the means were compared using Duncan's multiple range test at $p \leq 0.05$.

Results

Data in (table 1) showed higher germination 100 % at (0, 100 and 125) seconds of laser treatment respectively compare to lowest germination% rate 40 at (50) seconds. Also, results showed that laser treatments of (75,100 seconds) were significantly superior in giving highest number of shoots. Explants-1 with average of (3.63 and 3.67). As for the effect of laser on shoots height (cm), data showed that (75,100 seconds) were significantly superior which gave (7.3 and 7.47cm). Finally, laser treatments were also affected positively on number of leaves. shootlets-1 at (75,100 sec) which reached (6.53 and 7.70), (Fig 2).

Table1: *In Vitro* effect of laser exposure (seconds) on seeds germination % and some morphological traits of cabbage

Duration of laser treatment Sec	seeds germination %	number of shoots. Explants-1	shoots height (cm)	number of leaves. shootlets-1
0	100 a	0.4 b	3.07 bc	3.13 b
25	53.3 c	1.37 b	1.73 c	0.87 c
50	40 d	1.77 b	5.4 b	4.23 b
75	86.7 b	3.63 a	7.3 a	6.53 a
100	100 a	3.67 a	7.47 a	7.70 a
125	100 a	0.4 b	1.7 c	1.03 c

*Each value represent mean of three replicates
** Different letters refer to significant differences at p≤0.05 in Duncan's Multiple Range Test

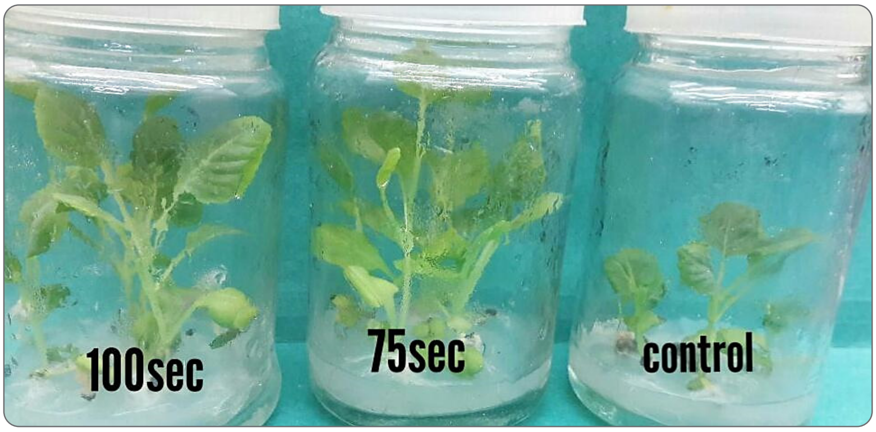

Fig 2: Irradiated seeds germination on MS media

For callus fresh weight and dry weight, data in (table 2 and 3), showed that a significant differences were found among the idividual parameters for example, at 125 seconds higher callus fresh water (FW) and dry water (DW) reached (167.73 mg and 20.43 mg) respectively. While maximum callus FW and DW were (188.12 mg ,29.98 mg) at 40 $g.l^{-1}$ of mannitol stress.

For the interaction between laser tratments and mannitol concentration, data in (table 2 and 3), showed that highest callus FW (232.87mg) in combination of (125seconds. + 40 $g.l^{-1}$) while higher callus DW was found at (25, 50 and 125seconds. in combination with 40 $g.l^{-1}$) namely (35.11, 33.6 and 33.95 mg) respectively.

Table 2: *In Vitro* effect of laser exposure (seconds) and mannitol stress on callus F.W. (mg)

Laser treatment Seconds	Mannitol $g.l^{-1}$			Mean
	0	40	60	
0	123.6 h	147.27 f	101.88 j	124.25 e
25	127.56 gh	158.85 e	105.58 ij	130.66 d
50	124.22 h	174.91 d	105.10 ij	134.74 d
75	135.55 g	200.21 c	112.83 i	149.53 c
100	144.15 f	214.63 b	110.30 ij	156.36 b
125	165.3 e	232.87 a	105.01 ij	167.73
Mean	136.73 b	188.12 a	106.78 c	

*Each value represent mean of three replicates
** Different letters within individual factors or between their interactions refer to significant differences at p≤0.05 in Duncan's Multiple Range Test.

Table 3: *In Vitro* effect of laser exposure (seconds) and mannitol stress on callus D.W. (mg)

Laser treatment Seconds	Mannitol $g.l^{-1}$			Mean
	0	40	60	
0	10.7 d	27.67 b	0.09 e	12.82 c
25	13.12 d	35.11 a	0.05 e	16.09 b
50	15.06 d	33.6 a	0.08 e	16.36 b
75	20.6 c	22.14 c	0.09 e	14.28 bc
100	22.22 c	27.42 b	0.11 e	16.58 b
125	27.6 b	33.95 a	0.09 e	20.43 a
Mean	18.22	29.98	0.09	

*Each value represent mean of three replicates
** Different letters within individual factors or between their interactions refer to significant differences at p≤0.05 in Duncan's Multiple Range Test.

Discussion:

Laser stimulation of plants is a physical phenomenon that depends mainly on the ability of cells to absorb and store the energy projected onto them (Gladyszewska, 2011, Sacala *et al*., 2012). The same thing happens to seeds, as they need light as a source of energy, which they convert into chemical energy later to be used for growth and development (Dinoev *et al*., 2004; Chen *et al*., 2010). Several vegetable and grain seeds were subjected to irradiation with different types of lasers to improve plant production in terms of quantity and quality (Costilla-Hermosillo *et al*., 2019). Research works in the field of environmental physics have demonstrated that biophysical techniques employed in agriculture can ensure average increases in seed germination of 20-35%, in root mass up to 24%, in leaf/stem mass between 10 and 45%, increased yield in the ranges of 10-50%, compared to plants grown under normal conditions, as well as increased resistance against diseases and pests (Hernandez *et al*., 2010; Pietruszewski and Kania, 2010; Aladjadjiyan, 2012; Hassanien *et al*., 2014).

Concerning to the results in (table 1) a gradual reduction in % germination when exposed to (25, 50 and 75 seconds) and remarkable increase at (100 and 125 seconds). The beneficial effect of Nd:YAG (green laser) irradiation with duration of exposure of 100 and 125 seconds, induced seeds germination with 100%. These results agreed with those reported in the literatures, supporting the fact that seed irradiation leads to acceleration of germination (Hernandez *et al*., 2010; Aladjadjiyan, 2012.; MOȘNEAGA *et al*., 2018). In addition to the possibility of increasing enzymatic activity, which in turn leads to stimulation of the cellular content of GA3, as it is important in cell elongation, and the increase of this hormone leads to the production of certain enzymes that have a role in weakening the cell wall in general, leading to faster germination (Osman *et al*., 2009 and Aguilar *et al*., 2015). Also, there was an agreement with Grishkanich *et al*., 2016, who used λ=532nm on Larch to enhance seeds germination. Their results showed that the germination% was higher in treated seeds than those of control treatment. According to Aladjadjiyan (2007) and Hernández-Aguilar *et al*., (2008), the inducing result is due to an extra increment in the energy of seed that's called bioplasm; that's why, elevation the energy ability of this bioplasm leads to elevate the stimulation process for the seed to germinate. Furthermore, (75 and 100 s.) caused significant increase in shoot multiplication/ no., shoots height, and number of leaves of *B. oleracea* var *capitata* when compared with control.

Laser light can be used as a biostimulator of seeds, seedlings and plants if it is used at low intensity (Hernandez *et al*., 2008b; Skvarko and Pochynok, 2010). Some studies on few species of Brassicaceae family had shown the positive effect of laser treatment, such as the exposure *of Isatis indigotica* to 633nm (He-Ne) for 5min could elevate activity of enzymes, accelerate physiological mechanisms in plant and increase plant growth after being exposed to UV-B light (Chen, 2008), *Brassica napus* L. when exposed to 532, 632 and 980 for 3 minutes showed positive changes in the proline content (Ashrafijou *et al*., 2010). On the other hand, the irradiation of *Triticum durum* with 532nm laser could be used to sterilize wheat seed and improve its growth and development (Rassam *et al*., 2012). There is difficulty in comparing the current results with the results of other researches, due to the fact that few studies have been conducted regarding the effect of laser light on physiological indicators related to plant productivity. Our results were agreed with Hasan *et al*., (2020) whose study found that seeds pretreated with green laser 532nm significantly increased the germination and growth parameters of Maize seeds, the best plant height and harvest index were reported when seeds treated with 125 seconds of green laser, as well as, best seed yield, harvest index, and yield efficiency when seeds treated with 85 seconds of green laser. In addition, Dobrowolski *et al*., 2012, stated that when laser with a wavelength of 532 nm and 25 mW of power was applied to soybean seeds, early germination was recorded in the irradiated seeds when compared to the control group.

In the current study, the seeds were exposed to low-intensity laser radiation for relatively short times, measured in seconds, because when exposed to laser for longer periods of time, significant damage could occur in the structure and composition of the seeds. Besides, the laser stimulation process is a physical phenomenon that includes both the absorption and storage of radiation energy by plant cells and tissues. The same phenomenon can be observed in the case of irradiation of seeds, where they first absorb the energy and then later convert it into chemical energy for the purpose of later use for growth (Abdullateef and Osman, 2011). The callus is a tissue with irregular cells of little differentiation. Its cells are of a parenchymatous nature and growth regulators play a major role in its formation, as they are added to the nutrient medium prepared for callus development (Bhojwani and Razdan, 1983). In order to obtain plant varieties with good tolerance to water stress, in addition to studying the tolerance mechanisms of these crops, various techniques of valuable importance are used in tissue culture laboratories for this purpose (Revilla and Canal, 1999). Such techniques play an important role in decreasing environmental variations as a result to defined media, controlled conditions and homogeneity of stress application.

Conclusion

The present study showed the importance role of both laser radiation and drought stress on cabbage morphological and physiological characters *In vitro*. Results revealed that higher germination 100 % at (0 and 100 and 125 seconds) of laser treatment. Whereas (75 and 100 seconds) were superior in giving highest number of shoots. Explants^{-1}, shoots height and number of leaves. Concerning the effect of laser treatments in combination with mannitol osmotic stress, the results clarified that the highest callus fresh weight was (232.87 mg) at 60 $g.l^{-1}$ of mannitol at 125 seconds of laser treatment, while 40 $g.l^{-1}$ of mannitol in combination with (25, 50 and 125 seconds) recorded the highest callus dry weight (35.11, 33.6 and 33.95 mg) respectively.

Further studies need to confirm if there is a mutation or change at DNA level by using DNA markers.

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