Effects of Laser Biostimulation on Germination of *Acacia farnesiana (L.)* Willd.

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Abstract

***Acacia farnesiana (L.)* Willd. is a very important multipurpose species in Egypt and many other countries. It is valued as forestry and as an ornamental tree. Its flowers produce a perfume called "cassie" extensively used in European perfumery. The present study aims to the determination of the effect of helium-Neon (He-Ne) light irradiation on accelerating the germination of *A. farnesiana* seeds, which have dormancy due to impermeability of seed coat of water. The seeds were exposed to He-Ne laser light for 1, 3, 5, 7, and 9 minutes with different irradiance 0.03, 0.20, 0.61, 1.14, and 1.70 W/cm^2. Gibberellic acid (GA), indole-acetic acid (IAA), abscisic acid (ABA) as well as phenols were determined after 48h in treated and untreated seeds. Thin sections in the testa were also made. Germination indices: the time to germinate, germination period, germination percentage and germination speed were determined.**

The results showed that irradiation by the laser beam of the seed coat, (testa or pericarp, the envelope enclosing the seed embryo), succeeded in perforating the seed coat at one or more positions. Generally, an increase in irradiance and exposure time of He-Ne laser light irradiation increased levels of endogenous promoting substances (GA and IAA), and decreased levels of endogenous inhibiting substances (ABA and phenol) in seed coats. We conclude that irradiance of seeds with He-Ne laser light at 1.70 W/cm^2 for 9 min. significantly affected germination indices when compared with control seeds.

INTRODUCTION

Acacia farnesiana Willd., is a thorny bush or small tree. The species has a pan tropical distribution incorporating northern Australia and southern Asia (Clarke et al., 1989).

Acacia farnesiana is a very important multipurpose species. It is valued as a forestry and ornamental tree 'Descriptors: soil conservation; erosion control; shelterbelts; windbreaks; hedges; amenity; and ornamental'. It is currently extremely popular in the local landscape industry. In addition, its flowers produce a perfume called "cassie" extensively used in European perfumery (CAB International, 2000). Different parts of the plant have been used for the treatment of various ailments (Sahu et al., 1998). Previous chemical investigation on the flowers and pods of the plant have revealed the presence of four homoterpene lactones, flavone glycosides and several polyphenolic compounds (El Sissi et al., 1974). Its leaves are also consumed by livestock and white-tailed deer (Ramirez et al., 1995).

Previous studies indicated that seeds of *Acacia farnesiana* are dormant due to impermeability of seed coat of water (Cervantes et al., 1996; Aref, 2000). It has been confirmed that the suitable dose of He-Ne laser irradiation improved germination capacity and enzymatic activities of plant seeds by breaking their dormancy (Anghel et al., 2000; Herandez et al., 2006).

The aim of the present study was to evaluate seed germination after application of the presowing He-Ne laser biostimulation of *Acacia farnesiana* seeds. In addition, to determine the optimal dose of radiation that could positively affect the germination kinetics of *Acacia farnesiana.*

MATERIALS AND METHODS

This study was conducted at the Experimental Laboratory of the Natural Resources Department, Institute of African Research and Studies, and the National Institute of Laser Enhanced Science, Cairo University, Giza, Egypt, during the two successive seasons of 2008 and 2009.

On the first of May 2008 and 2009 (in the first and second seasons, respectively) ripe fruits were collected from *Acacia farnesiana* trees grown in Al-Wahaat El-Baharya, 6 October Governorate, Egypt. Pods were crushed to release the seeds, which were tested for germination.

Treatments

On the first of June, in both seasons, seeds from the crushed pods were pre-treated with He–Ne laser (Model No.: PM450B, Serial No.: 865, wavelength 632.8 nm). Seeds were divided into five groups according to the exposure times to laser (1, 3, 5, 7, and 9 min.), and irradiance (0.03, 0.20, 0.61, 1.14, and 1.70 W/cm^2). As a control, untreated seeds were used.

This experiment was factorial (6 exposure time treatments (including the control) × 5 irradiance treatments), and conducted using a randomized complete design with three replicates for each treatment as well as the controls, and 60 seeds were used in each treatment during germination trials.

Germination trials were conducted in 9-cm sterile petri dishes lined with two Whatman No.1 filter papers and moistened with 10 ml of sterile distilled water to ensure adequate moisture for the seeds. Then petri dishes were covered with lids, but not sealed, and were maintained in incubator (Memmert, Model, ICP 400-800, made in Germany) at 30/25°C day/night, 12/12 h light/dark (Duke, 1981). Germination counts were monitored every two days for 80d. Germination was considered when the radical was visible (Hardegree and Emmerick, 1990).

At the end of the experiment (1st August 2008 and 2009, in the two seasons, respectively), germination indices were determined: time to germination as the time for the first germination to appear (T.G.), germination period as the time between the first germination and the end of germination (G.P.) according to Berrie (1985), germination percentage was calculated according to the equation of ISTA (1999):

$$\text{Germination percentage} = \frac{\textit{No. of germinated seeds}}{\textit{Total No. of seeds sown}} \times 100$$

Germination speed (G.S.) was also calculated using the following Equation of Maguire (1962):

GS = No. of seeds germinated/Days of first count +---+ No. of seeds germinated/Days of final count.

In addition, chemical analysis of untreated (control) and treated seeds were conducted after 48h. to determine the three plant internal hormones indole acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA) according to the method described by Du and Xu (2000), and total soluble phenols by using Folin-Ciocalteu method as mentioned (Meda et al., 2005). Thin sections also, were made in the testa of untreated and treated seeds.

The germination data and chemical analysis and were subjected to statistical analysis of variance. The means were compared using the "Least Significant Difference (L.S.D.)" test at the 5% level, as described by Little and Hills (1978).

RESULTS AND DISCUSSION

Effect of Different Irradiance Treatments and Exposure Times on

1. Seed Coat Anatomy. Marked differences in perforates were seen as a result of the different irradiance treatments of He-Ne red laser beam and exposure times on the seed coats anatomy of *Acacia farnesiana* (Fig. 1 a-f). Under the lowest irradiance (0.03 W/cm^2) for 1 min., the anatomy of seed coats was poorly affected, which had single perforated area (Fig. 1b). Meanwhile, the seed coats which irradiated at 1.70 W/cm^2, for 9 min. had the total perforated area in case of plural perforation (Fig. 1f). The main feature of the effects of laser beam which noted with the electron microscope (Fig. 1b-f), was only at the uppermost surface of seed coat. These results are in agreement with the findings of Zorov et al. (1985) on conifer seeds.

From the above mentioned results, it can be concluded that water impermeability of *Acacia farnesiana* seeds could be solved, by allowing a laser beam to irradiate the seeds. Kapelev (I989) and Ouf and Abdel-Hady (1999) indicated that the absorbed energy of He-Ne laser causing perforate at one or more positions or removing only the uppermost surface of the seed coat (testa or pericarp as the structure enclosing the seed embryo), then the water absorption and gas exchange increased, as a result of such treatment.

2. Endogenous Hormones and Phenols Contents. Data illustrated, (Fig. 2 a-d) showed that, in the two seasons, the level of endogenous hormones: GA_3 , IAA, and ABA, as well as, total soluble phenols in seed coats of *Acacia farnesiana* were significantly affected by irradiance treatments as compared to the control, regardless of the effect of exposure time treatments. Irradiated seeds at 1.70 W/cm^2 resulted in the highest level of endogenous contents of GA_3 and IAA followed by seeds irradiated at 1.14, 0.61, 0.20, 0.03 W/cm^2, and untreated seeds. Opposite trend was recorded for endogenous ABA and phenols contents, in both seasons. Such results are in agreement with the findings of Kamiya et al. (1999) on lettuce seeds.

As shown in (Fig. 2a-d) the lowest contents of GA_3 (2.36 and 2.51 mg/100 g F.W., respectively) and IAA (1.89 and 2.94 mg/100 g F.W., respectively) as well as, the highest contents of ABA (0.82 and 0.78 mg/100 g F.W., respectively) and phenols (8.48 and 6.42 g/100 g F.W., respectively) were found in untreated seeds (control), whereas the highest contents of GA_3 (37.90 and 43.07 mg/100 g F.W., in the first and second seasons, respectively) and IAA (28.72 and 32.22 mg/100 g F.W., in the first and second seasons, respectively) as well as, the lowest contents of ABA (0 mg/100 g F.W., in both seasons) and phenol (0.65 and 0.45 g/100 g F.W., in the first and second seasons, respectively) were found in plants exposed for 9 min., regardless of the effect of irradiance treatments.

The data illustrated, in the two seasons, (Fig. 2a-d) revealed that within the treatments the highest contents of GA_3 and IAA as well as, the lowest significant contents of ABA and phenol in the seed coats were obtained as a result of irradiation the seeds with He Ne laser at 1.70 W/cm^2 for 9 min.

The changing in endogenous hormones: GA_3, IAA, and ABA, as well as, total soluble phenols as a result of He-Ne laser treatments was explained by El-Tobgy et al., (2009) they confirmed that the red light laser can induce GA_3 ß hydroxylase gene (S_3 h_1 expression) which promotes the complex cycle of GA_3 formation, while the polychromatic light (sunlight) cannot induce this effect. Raven et al. (1992) and Davies (1995) reported that the GA mainly to break seed dormancy, cell division (with IAA) induces and formation of protolytic enzymes that would be expected to release is tryptophan precursor of Indol acidic acid (IAA), and overcome the germination inhabitation of ABA which has some effect on testa or seed coat growth characteristics, including thickness and maintaining dormancy (Mauseth, 1991; Salisbury and Ross, 1992; Tsai et al., 1997).

3. Germination Indices. The data presented in Tables (1-4) showed that, in both seasons, germination indices of *Acacia farnesiana* were significantly affected by He–Ne laser irradiance treatments as compared to the control, regardless of the effect of exposure time treatments. Irradiated seeds at 1.70 W/cm^2 resulted in a shorter time to the starting of germination and the germination period, as well as significantly increased germination percentage and germination speed, followed by the irradiation of the seeds at 1.14, 0.61, 0.20 and 0.03 W/cm^2, with significant differences among themselves. Such results were similarly reported by Podlesny (2002) on the faba bean *(Vicia fabaminor),* and Gladyszewska (2006) on wheat. In addition, Muszyñski and Gladyszewska (2008) on radish *(Raphanus dativus L.).*

Data presented in Tables (1 and 4) revealed that increasing exposure times had an effect on germination indices of *Acacia farnesiana,* regardless of the effect of irradiance treatments. In both seasons, germination percentage and germination speed were increased as the exposure time was increased for 0, 1, 3, 5, 7 or 9 min. but it had an adverse effect on the time to germination, and germination period. These results are in agreement with the findings of Abdel-Fatah (2005) on sage plant *Salvia officinalis,* Junlin et al., (2007) on Chinese pine (Shaanxi) and Abu-Elsaoud et al., (2008) on four wheat *(Triticum aestivum).*

Regarding the interaction between the irradiance treatments and the exposure times, the data recorded, in the two seasons, (Table, 1) showed that highest value of germination percentage and germination speed, as well as, lowest value of time to germination and germination period were obtained as a result of irradiated with He-Ne laser at 1.70 W/cm^2 for 9 min.

Many researchers reported that the influence mechanism of low power laser, especially the laser of visible wavelength is most likely attributed to its light effect and electromagnetism effect. So, the seeds which pretreated with laser were notably higher in enthalpy change (DH) and have to absorb more energy from the surrounding than that of the control during the germination process because laser broke the kinetic equilibrium of germination seeds and enhanced the internal energy of seeds. Consequently, the biochemistry and physiology metabolisms of the seeds pretreated with laser were accelerated (Han et al., 2002; Rubinov, 2003; Salyaev et al., 2003; Chen et al., 2005a,b; 2005b; Samuilov and Garifullina, 2007; Wu et al., 2007).

CONCLUSIONS

1. It is important to use and compare several common germination indices in order to examine their abilities in description of the effect of external stimulus of laser irradiation on seeds germination process.
2. Irradiation the seed of *Acacia farnesiana* with He-Ne laser has a significant effect of germination indices.
3. The optimal dose of radiation that could positively affect the germination kinetics of *Acacia farnesiana* seeds was found at 1.7 W/cm^2 for 9 min.

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Tables

Table 1. Effect of different irradiance treatments and exposure times of He-Ne laser on germination percentage (%), time to germination (day), germination period (day), and germination speed (seed d^{-1}) of *Acacia farnesiana* during the two seasons of 2008 and 2009.

Irradiance (I.) (W/cm^2)	1st Season							2nd Season						
	Exposure times (E.T.) (min.)						Mean	Exposure times (E.T.) (min.)						Mean
	0	1	3	5	7	9		0	1	3	5	7	9	
	Germination Percentage (%)													
0.03	25.00	31.67	38.00	46.67	63.33	71.67	**46.06**	26.67	36.67	48.33	55.00	73.33	83.33	**53.89**
0.20	25.00	36.67	46.67	53.33	73.33	83.33	**53.06**	26.67	40.00	55.00	63.33	76.67	88.33	**58.33**
0.61	25.00	40.00	53.33	61.67	78.33	91.67	**58.33**	26.67	46.67	56.67	73.33	88.33	95.00	**64.44**
1.14	25.00	48.33	54.67	66.67	80.00	96.67	**61.89**	26.67	48.33	65.00	76.67	93.33	98.33	**68.06**
1.70	25.00	50.00	58.33	76.67	86.67	100.00	**66.11**	26.67	53.33	71.67	81.67	96.67	100.00	**71.67**
Mean	**25.00**	**41.33**	**50.20**	**61.00**	**76.33**	**88.67**	**---**	**26.67**	**45.00**	**59.33**	**70.00**	**85.67**	**93.00**	**---**
LSD (0.05)	**I= 2.81**		**E.T.= 4.87**		**I. X E.T.= 6.88**			**I= 1.93**		**E.T.= 2.81**		**I. X E.T.= 4.74**		
	Time to Germination (day)													
0.03	19.33	18.00	17.33	14.00	13.33	10.67	**15.44**	16.67	15.33	14.00	12.67	10.00	8.00	**12.78**
0.20	19.33	16.67	15.33	11.33	10.67	7.33	**13.44**	16.67	14.67	12.67	10.67	8.00	6.67	**11.56**
0.61	19.33	16.00	14.67	12.00	8.67	6.00	**12.78**	16.67	13.33	10.00	8.67	7.33	4.00	**10.00**
1.14	19.33	13.33	10.00	9.33	7.33	4.00	**10. 56**	16.67	12.67	8.67	7.33	6.67	3.33	**9.22**
1.70	19.33	12.67	8.00	7.33	4.67	2.67	**9.11**	16.67	10.00	6.67	6.00	4.00	2.00	**7.56**
Mean	**19.33**	**15.33**	**13.07**	**10.80**	**8.93**	**6.13**	**---**	**16.67**	**13.20**	**10.40**	**9.07**	**7.20**	**4.80**	**---**
LSD (0.05)	**I= 0.77**		**E.T.= 1.88**		**I. X E.T.= 1.89**			**I= 0.67**		**E.T.= 1.07**		**I. X E.T.= 1.63**		
	Germination Period (day)													
0.03	54.67	48.67	44.67	42.67	38.00	34.67	**43.89**	52.00	46. 67	42.00	37.33	35.33	31.33	**40.78**
0.20	54.67	45.33	43.33	40.00	34.67	30.67	**41.44**	52.00	44.00	39.33	36.00	31.33	28.00	**38.44**
0.61	54.67	42.67	40.00	38.67	30.67	28.67	**39.22**	52.00	42.00	38.67	34.00	28.67	25.33	**36.78**
1.14	54.67	41.33	35.33	33.33	28.00	25.33	**36.33**	52.00	39.33	36.00	31.33	26.67	23.33	**34.78**
1.70	54.67	38.67	30.67	29.33	27.33	22.67	**33.89**	52.00	36.00	32.00	26.67	24.00	20.00	**31.78**
Mean	**54.67**	**43.33**	**38.80**	**36.80**	**31.73**	**28.40**	**---**	**52.00**	**41.60**	**37.60**	**33.07**	**29.20**	**25.60**	**---**
LSD (0.05)	**I= 1.20**		**E.T.= 2.86**		**I. X E.T.= 2.94**			**I= 1.25**		**E.T.= 2.95**		**I. X E.T.= 3.07**		
	Germination Speed (seed d^{-1})													
0.03	3.18	6.39	7.08	8.99	11.71	13.71	**8.51**	4.23	9.45	11.19	19.86	20.17	23.16	**14.68**
0.20	3.18	7.74	9.27	17.57	16.43	26.69	**13.48**	4.23	11.37	14.24	20.27	23.44	28.49	**17.01**
0.61	3.18	10.20	12.23	18.64	21.75	28.38	**15.73**	4.23	12.24	15.32	23.58	24.89	30.44	**18.45**
1.14	3.18	13.04	16.40	22.71	23.57	35.24	**19.02**	4.23	13.14	17.21	25.18	26.42	39.18	**20.89**
1.70	3.18	15.13	18.40	23.38	24.92	47.26	**22.05**	4.23	16.85	19.70	27.13	28.34	49.11	**24.23**
Mean	**3.18**	**10.50**	**12.68**	**18.26**	**19.68**	**30.26**	**---**	**4.23**	**12.61**	**15.53**	**23.21**	**24.65**	**34.08**	**---**
LSD (0.05)	**I= 0.89**		**E.T.=1.04**		**I. X E.T.= 2.17**			**I= 0.85**		**E.T.= 1.09**		**I. X E.T.= 2.08**		

Figures

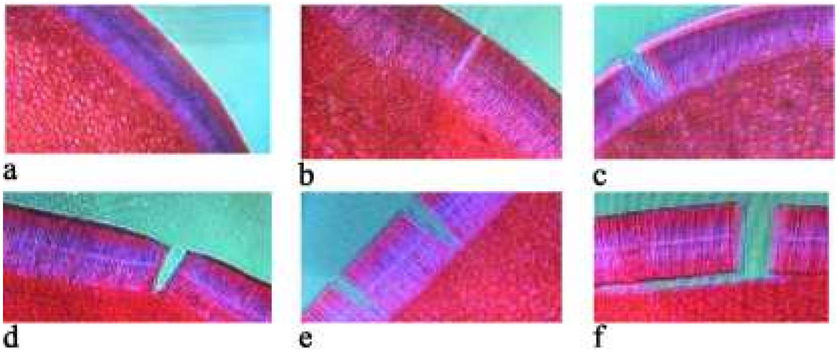

Fig. 1. Seed coat anatomy of *Acacia farnesiana* as affected by exposure time (9 min.) and different irradiance of He-Ne laser treatments: a- untreated seed, b- 0.03, c- 0.20, d- 0.61, e- 1.14 and f- 1.70 W/cm^2.

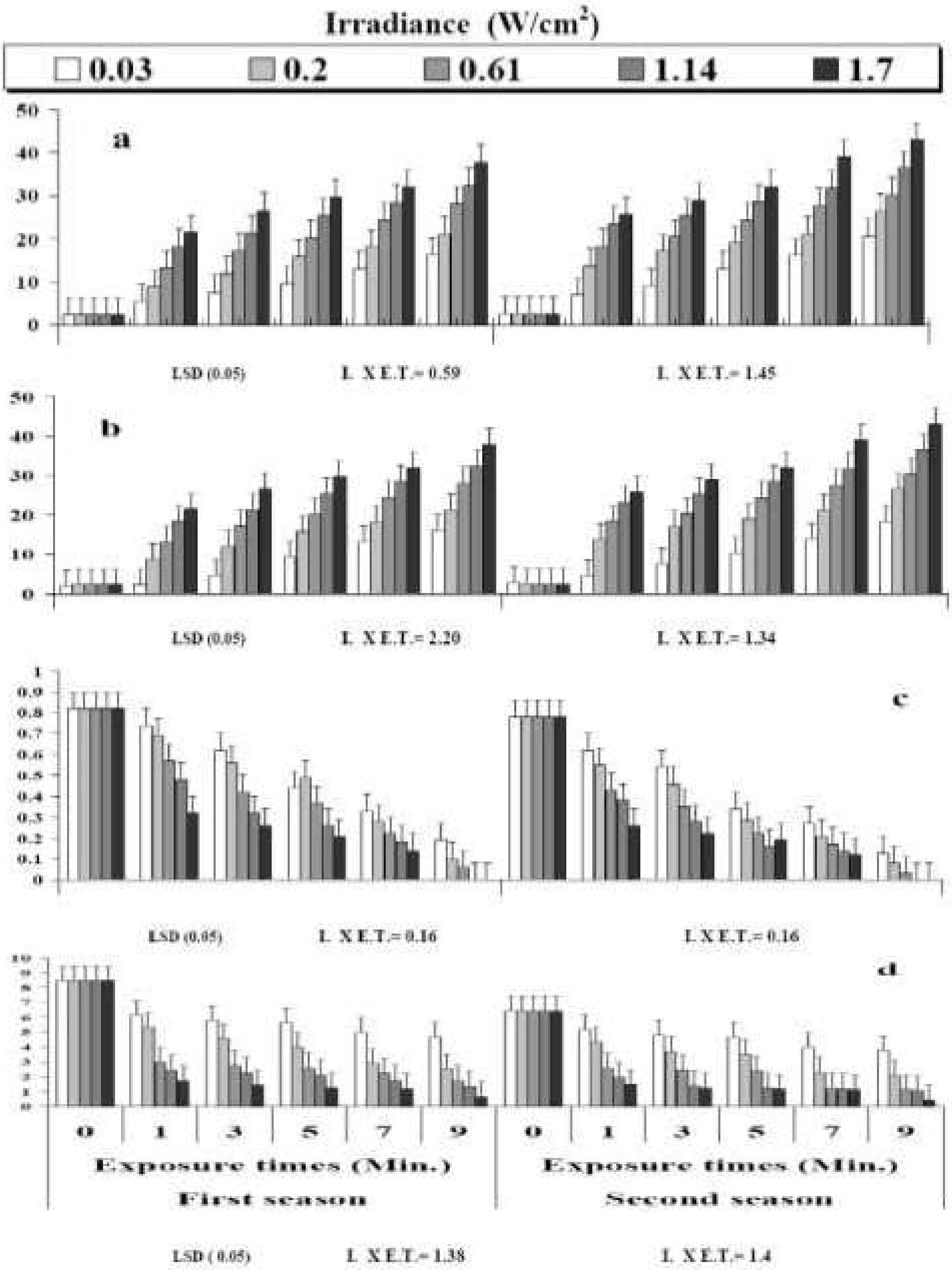

Fig. 2. Effect of different irradiance (I.) treatments and exposure times (E.T.) on endogenous hormones (a- GA, b- IAA, c- ABA (mg/100 g F.W)) as well as (d) phenols (g/100 g F.W.) in seed coat of *Acacia farnesiana* during the two seasons of 2008 and 2009.