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INFLUENCE OF RED LASER RADIATION ON THE VIGOUR OF TOMATO SEEDS AFFECTED BY AGING

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ABSTRACT

The loss of vigour in tomato seeds (*Solanum lycopersicum* L.) due to aging is a normal condition. This study provides a future reference in regard to the possible applications pre-sowing of red laser radiation treatments on tomato seeds to improve the vigour of the aged seeds. The objective of the study was to analyse the effects of laser radiation on aged tomato seeds, on the rate of germination and growth. It is hypothesized that at least one treatment of red laser radiation could improve the seed vigour. Two groups of seeds were used, one with 11 years of aging and another with seeds aged 11 years plus 24 h of artificial aging. For the radiation, a red laser diode of 660 nm was used with a potency of 100 mW, at a radiation density of 0.2 mW cm^{-2}, eight exposure times to the laser radiation and a control. The data were subjected to an analysis of variance and the means were compared with the Tukey test (HSD; $p \leq 0.05$). In seeds with 11 years of aging, the laser treatment at 120 s improved seed vigour by 125 %, resulting from an increase in the growth of the radicle (167 %, $p \leq 0.05$) compared to the control (100 %). On the other hand, the laser treatment at 60 s improved the tolerance to stress from artificial aging, the vigour index increased 143 % compared to the control (100 %), as a result of an improvement in germination of 162 % and in the hypocotyl growth (130 %, $p \leq 0.05$), compared to the control (100 %). Red laser radiation on tomato seeds affected by natural or artificial aging can influence the processes of photo morphogenesis and increase seed vigour.

Keywords: tomato seeds; laser radiation; improvement of vigour; stimulus in length of radicle and hypocotyl, germination.

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INTRODUCTION

The tomato (*Solanum lycopersicum*), a member of the Solanaceae family, is among the most cultivated vegetables in the world and has high economic and nutritional value. In 2019, the area sown with tomato throughout the world was 5 million hectares, with a yield of 180 million tons (FAOSTAT, 2019).

Vigorous seeds are a key element for the success of a crop (Finch-Savage and Bassel, 2016). Seed vigour is a complex property that determines potential for emergence and rapid and uniform development under a wide range of field conditions; it depends fundamentally on the capacity to resist extended storage and the harmful effects of aging (Rajjou *et al*., 2012). As storage time of the seeds increases the seeds age, which provokes the physiological deterioration of the seeds (Suresh *et al*., 2019). Vigour also refers to the maximum period of time during which the seeds maintain viability for germination (Ku *et al.* (2014).

For agricultural use, the aged seeds lose biological functions that decrease their viability over time, which is manifested in a poor establishment of the crop in the field (Jacob *et al*., 2016). The main impacts of the variation in seed vigour are expressed through a direct negative effect on the emergence of the seedlings, and therefore, an indirect effect on yield (Tekrony and Egli, 1991).

The artificial aging of seeds, with high temperature and humidity, imitate long-term natural aging; these conditions accelerate the metabolism and induce the accumulation of harmful alterations associated with aging (Fleming *et al*., 2018). The artificial aging technique in seeds reproduces natural aging because, in natural and artificial aging, the same physical and chemical effects occur (Suresh *et al*., 2019).

An important factor in the establishment of a crop is the germination of the seed. Germination is controlled by internal and external factors, such as genetics, seed structure, seed chemistry, moisture and temperature (Lei *et al*., 2013). The capacity of a plant to maximize germination, growth and photosynthetic productivity of the seeds depends on the capacity to detect, evaluate and respond to the quality, amount and direction of the light (Swathy *et al*., 2021).

Some researchers utilize physical methods such as laser radiation to improve the processes of germination, growth and productivity in the plants (Ćwintal and Dziwulska-Hunek, 2013; Prośba-Bialczyk *et al*., 2013). In *Vigna radiata,* the pre-treatment of seeds with laser radiation at λ = 632.8 nm and λ = 488 nm improved the length of the hypocotyl by 22.5 and 28 % in the radicle compared to the control (Janayon and Guerrero, 2019). Swathy *et al.* (2021) applied treatments with He-Ne radiation in eggplant seeds observing a substantial improvement in the germination index, germination time and vigour index of the seed in regard to the control. The physical treatments such as laser radiation in seeds prior to sowing are environmentally safe, because only biochemical and physiological processes in the seed are modified, thus accelerating the emergence of the seedlings and improving plant development (Podlesny *et al*., 2012).

The use of laser bio-stimulation to improve crops is a technique which is under development. The objective of this study was to analyse the effects of red laser radiation on tomato seeds affected by aging, on the characters of germination and initial growth of seedlings, with the aim of improving and preserving the vigour of the tomato seeds. It was hypothesized that at least one treatment of red laser radiation would improve the seed vigour.

MATERIALS AND METHODS

The project was developed in the bio-photonics laboratory of the Michurinsk State Agrarian University, located in the city of Michurinsk, Russia, during the month of July of 2019.

Details of the seed lot

Tomato (*Solanum lycopersicum*) seeds variety Orange were used. The seeds were supplied by Michurinsk State Agrarian University. At the start of the experiment the seeds had an age of approximately 11 years, during which they had been stored with a mean temperature of 19 ± 4 °C and a percentage of relative humidity of 25 ± 5 %. Seed selection was according to what is stipulated in the international rules for seed testing (ISTA, 2017).

Procedure for inducing artificial aging

In the procedure for inducing artificial aging, four groups of 250 selected seeds were taken and thermally treated at 45 ± 2 °C with a relative humidity of 100 ± 5 % during 24 h. The temperature was constant, and humidity was controlled according to what was described by Seyyedi *et al.* (2018) and Han *et al.* (2014). For this procedure a thermostat was used (TSO-1/80 SPU, Himmedservis, Russia). Prior to initiating the procedure, the temperature and percentage of humidity were verified, and the seeds were placed in Petri dishes with a layer of paper filter, then were placed in the thermostat. Next, the door of the thermostat was hermetically closed and maintained without light throughout the procedure (24 h).

Germination test

The germination test was done in Petri dishes with three layers of filter paper moistened with distilled water (Kornarzyński *et al.*, 2018). 25 seeds were used per Petri dish, with four replications for each treatment (Hadi *et al.*, 2018; Badran and Savin, 2018). The temperature of germination was around 22 ± 5 °C, and germination was with controlled light during the day and without light during the night (12/12 h).

Process of laser radiation of seeds

Prior to the radiation process, the seeds were submerged in demineralized water during 60 min. Next, the excess water was removed, and the seeds were dried with filter paper, and the laser radiation treatments were applied. For the radiation, an experimental laser diode module was used (Figure 1-2 A), with a potency of 100 mW and wavelength of 660 nm continuous mode, at power density in the irradiation plane of 0.2 mW cm^{-2}. The radiation density was verified prior to each treatment with a potentiometer Ophir model 7Z01560 made in Israel and a filter at 1 cm^{-2} (Figure 1-2 B). Eight exposure times of exposure to radiation are proposed (1, 7.5, 15, 30, 60, 120, 240 and 480 s) and two controls without treatment (control "C" and control plus artificial aging "C.T").

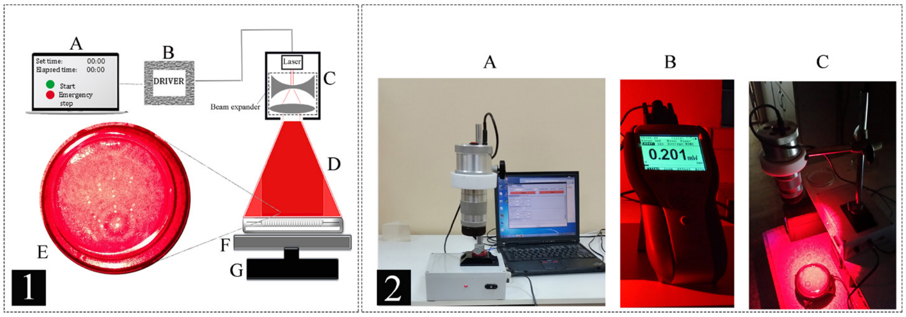

Figure 1. 1: Diagram of the radiation process. A: software to define and activate the time of laser radiation, B: driver, C: laser module, D: laser radiation 660 nm, E: seeds, G: motor with F: rotational surface. 2). A: laser module and control unit, B: meter of laser power density with filter of 1 cm^{-2}, C: radiation process.

During the process of laser radiation, the seeds were found in a Petri dish with a layer of filter paper, ordered within a circular surface of 9 cm diameter. To homogenize the laser radiation, they were placed on a stationary rotational surface and were uniformly accelerated (1.81×10^{-3} x*g*). Exposure time to the laser radiation was defined with a software that activates a driver B that turns on the laser C, the laser radiation D impact on the seeds E that were on a rotating surface F uniformly accelerated by a motor G, the exposure times were monitored with a processing unit A (Figure 1-1).
The values of the vigour index were calculated with the following equation (Mariappan *et al.*, 2014):

$$V = G(\%)*T$$

where: V is the vigour index, G is germination (%), T is total length (mm) of the seedling (hypocotyl plus radicle).

Germination

The percentage of germination was calculated based on the total number of seeds germinated at the end of the experiment and the values of the germination index were calculated with the following equation (Almutairi, 2016):

$$\text{Stimulus } (\%) = \left(\frac{G_F}{n}\right) * (100)$$

where: G_F is the total number of seeds germinated at the end of the experiment and n is the total number of seeds in the test.

Percentage of stimulus

The percentage of stimulus was calculated adopting the following equation:

$$\text{Stimulus (\%)} = \left(\frac{A_n}{A_c}\right) * (100)$$

where: Stimulus (%) is the stimulus produced by a given treatment, A_n is the mean value of a parameter in analysis produced by a given treatment, A_c is the mean value of a parameter in analysis of the group without treatment (control).

Analysis of the data

For the evaluation of the effects of stimulation provoked by the laser radiation, four factors were considered: germination, vigour, length of hypocotyl and length of radicle. The evaluation occurred 11 days after the start of the experiment. The software STATISTICA was used to process the data with ANOVA and the Tukey's test of multiple comparison of means (HSD; $p \leq 0.05$).

RESULTS AND DISCUSSION

Effects produced by laser radiation on seeds with 11 years of aging

Under natural conditions, the seeds stored over a long time are subject to aging. According to the rate and magnitude of aging, the vigour and viability of the seeds are negatively affected (Huang *et al.*, 2021). The use of physical methods such as red laser radiation is an option for increasing seed vigour (Mohammadi *et al.*, 2012); the effects produced will depend on the treatment applied.

The interaction among the seeds and the eight times of exposure to the radiation tested generated some significant effects ($p \leq 00.05$; Table 1). The treatments with 120 and 480 s accelerated the dynamic of germination (Figure 2), the fourth day of evaluation showed a stimulus in germination of 100 % for 120 s and 98 ± 2 % for 480 s compared to the control (0 %). On the other hand, at day 5 the difference was reduced to 4 %, which shows that the germination levels were not significantly affected, but the velocity of germination was improved.

The treatment at 120 s of laser radiation improved seed vigour by 120 % compared to the control (C, 100 %) and was the treatment with best results (Table 1).

Li *et al.* (2017) found that the red light (640 to 660 nm) promoted stem length, leaf area, saccharose, concentrations of soluble sugar and starch, as well as the number and volume of starch grains in the chloroplast of cotton (*Gossypium hirsutum*).

The stimulating effect of red laser radiation did not generate the same impact in the radicle and hypocotyl. With the treatment at 120 s of laser radiation, growth of the radicle was significantly affected ($p \leq 0.05$), generated an elongation in the length of the radicle in 167 ± 4 % compared to the control (100 ± 9 %), and in growth of hypocotyl

Table 1. Germination, vigour, growth of radicle and hypocotyl (mean ± EE) of seeds of tomato (*Solanum lycopersicum*) variety Orange with 11 years of aging, after the treatments with red laser radiation.

T† (s)	G¶		V§		Rᵇ		H¤	
	Media (%)	Estímulo (%)	Media índex	Estímulo (%)	Longitud (mm)	Estímulo (%)	Longitud (mm)	Estímulo (%)
C	94 ± 3.4	100.0 ± 3.7	1963.1	100.0	7.47 ± 0.7	100 ± 9	13.41 ± 1.1	100 ± 6
1	96 ± 2.3	102.2 ± 2.4	2012.6	102.5	7.43 ± 0.5	99 ± 6	13.55 ± 0.8	100 ± 5
5	98 ± 1.2	104.3 ± 1.2	2219.4	113.0	8.35 ± 0.5	112 ± 6	14.29 ± 0.8	104 ± 4
15	98 ± 1.2	104.3 ± 1.2	2033.8	103.6	7.42 ± 0.5	99 ± 6	13.33 ± 0.8	104 ± 4
30	96 ± 0	102.1 ± 0	2239.3	114.1	11.77 ± 0.8	158 ± 7††	11.55 ± 0.8	86 ± 5
60	92 ± 0	97.9 ± 0	2061.7	105.0	9.23 ± 0.7	123 ± 8	13.18 ± 1	102 ± 6
120	100 ± 0	106.4 ± 0	2486.8	126.7	12.48 ± 0.5	167 ± 4††	12.39 ± 0.5	88 ± 3
240	98 ± 1.2	104.3 ± 1.2	2157.1	109.9	10.48 ± 0.7	140 ± 6††	11.56 ± 0.7	84 ± 5
480	100 ± 0	106.4 ± 0	2000.2	101.9	9.51 ± 0.5	127 ± 6	10.49 ± 0.6	75 ± 4

†T: time in seconds; ¶G: germination; §V: vigour; ᵇR: radicle; ¤H: hypocotyl; ††Statistically significant differences ($p \leq 0.05$).

no significant effect was generated. The diode laser radiation 660 nm did not show significant effects in short exposure times (Table 1). Therefore, in the second part of this study (seeds affected by natural aging plus 24 h of artificial aging) the radiation times of 1, 5 and 15 s were discarded. In this first stage (seeds with 11 years of aging), the stimulus provoked by the treatments with laser radiation in the growth factor caused a multimodal behaviour with three statistically significant values at 30, 120 and 240 s A maximum value was found at 120 s (Figure 3), after this maximum value the stimulus produced by the laser radiation decreased.

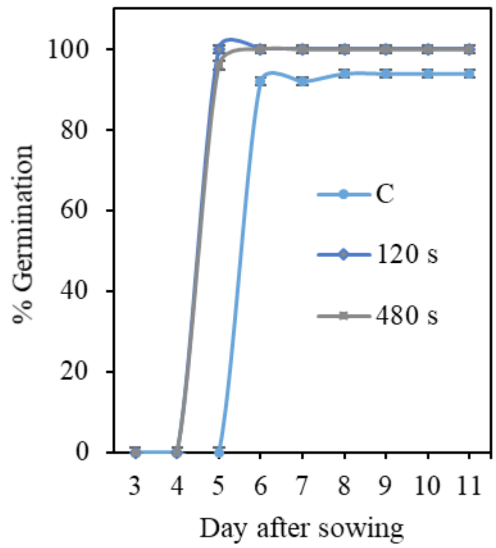

Figure 2. Stimulus in germination dynamic in tomato seeds (*Solanum lycopersicum*) variety Orange with 11 years of natural aging, radiated with red laser radiation (660 nm).

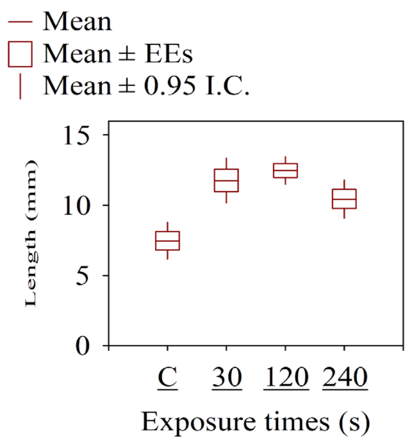

Figure 3. Effects on growth of hypocotyl and radicle of tomato plants (*Solanum lycopersicum*) variety Orange from seeds with 11 years of aging radiated with red laser radiation (660 nm). EE: Standard error; I.C., confidence interval. The data represented by C, 30, 120 and 240 s pertain to the part of the radicle.

Effects produced by laser radiation in seeds with artificial aging

In germination, the control (C.T.) showed a germination of 32 ± 4.6 %, the treatment with 60 s of laser radiation generated a strong stimulus in germination dynamic (Figure 4); and an improvement of 162 ± 8.9 % was expressed in the final germination compared to the control 100 % (Table 2).

In seed vigour the best results were obtained with the treatment 60 s of laser radiation, with a stimulation of 195 % ($p \leq 0.05$), compared to the control (C.T., 100 %; Table 2). On the other hand, the treatments with 30 and 120 s generated a significant deterioration in seed vigour of more than 55 % compared to the control (C.T., 100 %).

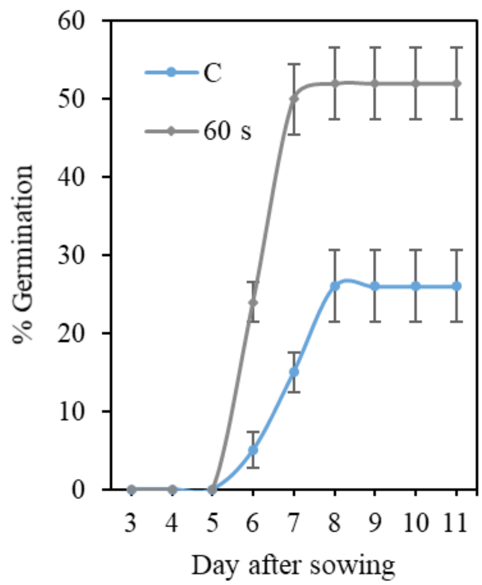

Figure 4. Stimulus in germination dynamic in tomato seeds (*Solanum lycopersicum*) variety Orange with 11 years of aging plus 24 h of artificial aging, radiated with red laser radiation (660 nm).

Table 2. Germination, vigour, growth of radicle and hypocotyl (mean ± EE) of tomato seeds (*Solanum lycopersicum*) variety Orange with 11 years of aging plus 24 h of artificial aging, after treatments with red laser radiation.

T† (s)	G¶		V§		Rᵖ		H¤	
	Media (%)	Estímulo (%)	Media índex	Estímulo (%)	Longitud (mm)	Estímulo (%)	Longitud (mm)	Estímulo (%)
C. T	32 ± 4.6	100 ± 14.4	944	100.0	16.2 ± 3.5	100.0	13.3 ± 3	100.0
C	96 ± 2.4	300 ± 2.5	5376	569.5	35.8 ± 1.8	221.0	20.2 ± 1.5	151.9
30	28 ± 6.9	87.5 ± 24.7	420	44.5	9 ± 2.2	55.6††	6 ± 1.9	45.1††
60	52 ± 4.6	162.5 ± 8.9	1840.8	195.0	18 ± 2	111.1††	17.4 ± 1.9	130.8††
120	20 ± 6.9	62.5 ± 37.7	292	30.9	9 ± 2.8	55.6††	5.6 ± 2	42.1††
240	36 ± 6.9	112.5 ± 9.5	738	78.2	13.5 ± 2.7	80.2	7.5 ± 1.9	56.4
480	36 ± 2.3	112.5 ± 6.4	802.4	106	14 ± 2.6	86.4	8 ± 1.6	60.2

†T: time in seconds; ¶G: germination; §V: vigour; ᵖR: radicle; ¤H: hypocotyl; ††Statistically significant differences ($p \leq 0.05$).

In the analysis of seedling growth, the treatment with 60 s of laser radiation showed the best results; in hypocotyl it generated a stimulus of 130.8 % and in radicle, 111 % compared to the control (C.T., 100 %; Table 2). The treatments with 30 and 120 s of laser radiation generated a decrease in growth of the seedling in radicle and hypocotyl ($p \leq 00.05$).

Yang *et al.* (2012) subjected wheat seeds to treatments with radiation UV-B, the UV-B treatment reduced the rate of germination and growth significantly ($p \leq 0.05$) in regard to the control, and afterwards they used red laser radiation to reactivate the seeds, they found that with red laser radiation treatments it is possible to mitigate the effects generated by the UV-B treatment by 15.4 % in germination and 60 % in growth.

Gao *et al.* (2018) affected seeds of wild *Arabidopsis thaliana* (ecotype Columbia-0) with UV-B radiation, and later evaluated the effects of red laser radiation, observing that the repair of the damage from UV-B of the seeds is activated with He-Ne laser due to the over-regulation of some genes that codify proteins sensitive to UB-B. This suggests that the 600nm laser radiation can serve as an option of improvement of vigour in seeds subjected to stress.

The treatments with artificial aging utilized in this study notably decreased the viability of the seeds; germination was reduced from 96 % to 32 %. The reduction in germination was accompanied by a delayed and unsynchronized germination.

The treatment with laser radiation of 60 s showed a stimulus in germination (162 %) regarding the control (C.T., 100 %), in growth of hypocotyl and radicle the effect was positive (111 %, radicle; 130 %, hypocotyl; Table 2) compared to the control (C.T., 100 %). An alternative and hypothetical explanation that requires evidence is that the red laser radiation improved the percentage of germination and growth of the seedlings.

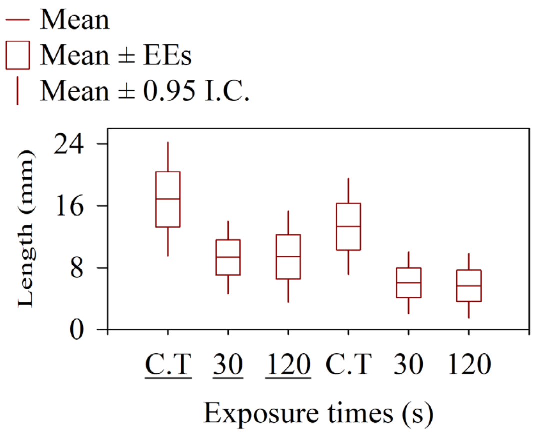

Figure 5. Representative effects ($p \leq 0.05$) in growth of hypocotyl and radicle of tomato seedlings (*Solanum lycopersicum*) variety Orange from seeds with 11 years of aging plus 24 h of artificial aging radiated with red laser radiation 660 nm. EE: standard error; I.C.: confidence interval. (The data represented by C.T., 30 and 120 s pertain to the radicle portion and C.T., 30, 120 s, to the hypocotyl).

The treatments with artificial aging (C.T.) caused a very large loss is seed vigour compared to the control (C) of around 470 % (Table 2). These results demonstrated that the treatment of artificial aging worked.

In terms of vigour in seeds affected by artificial aging, the treatment with 60 s was better (195 %) than the control (C.T., 100 %; Table 2). The treatments at 30 and 120 s of red laser radiation caused strong negative effects ($p \leq 0.05$) in growth of radicle and hypocotyl, which decreased the growth of the seedling (Figure 5). The treatment of artificial aging can cause a change in the sensitivity to red light of the photosensitive terminals which are present in the seeds.

The treatments of laser radiation gave evidence of positive effects for increasing the vigour of the seeds affected by artificial and natural aging. At the seedling level the effects are visible in morphological changes, while the magnitude and the effect will depend on the dose of radiation applied. It is important to find techniques of application of the radiation dose that help to define the synergy between the red laser radiation treatments and the tomato seeds, which in consequence positively affect seed vigour.

CONCLUSIONS

The characters of germination and growth in aged seeds of tomato variety Orange can be improved with red laser radiation treatment. The regeneration of aged seeds (11 years) was achieved from the synergy between the red laser radiation treatments and the photosensitive terminals of the seeds.

The treatments with red laser radiation can improve vigour of tomato seeds aged naturally or artificially. This study demonstrated the use of the red diode laser (660 m) as a potential tool for improving the germination and growth in tomato seedlings variety Orange with more than 11 years of natural aging.

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