**Manuscript type:** Original Research Article

LASER RADIATION TO STIMULATE TOBACCO GROWTH

Emilia Mihaylovaa\*, Teodora Pashovaa, Marina Drumeva-Yonchevab, Nelly Keranovac

aDepartment of Melioration, Land Management and Agrоphysics, Agricultural University of Plovdiv, Bulgaria,

bTobacco and Tobacco Products Institute, Plovdiv, Bulgaria,

cDepartment of Mathematics and Informatics, Agricultural University of Plovdiv, Bulgaria,

\*Corresponding author: Emilia Mihaylova

[emmihaylova@gmail.com](mailto:emmihaylova@gmail.com)

Agricultural University of Plovdiv, 12 Mendeleev Boulevard, 4000 Plovdiv, Bulgaria

**Novelty statement:** We report first results of the effect of laser irradiation of seeds before sowing. Three genotypes of Virginia tobacco, whose seeds were irradiated with a red laser (λ=655 nm, P=0,7 mW). It was found that pre-sowing laser irradiation of tobacco seeds with a red laser led to an acceleration of germination.

**Abstract**

During the last 50 years the progress of chemical technology provoked production revolution in the agriculture. Many chemicals are used for fertilizing crops, controlling pests and helping to develop a highly successful farm system. The long-term use of chemicals in agricultural production resulted in a decrease in plant resistance, plant yield and soil yielding capacity. The negative effects of chemicals on the agricultural produce and on the environment are commonly known. Therefore, many scientists believe that this century is the century of biophysical methods in agriculture. One biophysical method for plant growing stimulation is laser irradiation of seeds before sowing.

The subject of this study were three genotypes of Virginia tobacco, whose seeds were irradiated with a red laser (λ=655 nm, P=0,7 mW) with different duration (30 s, 60 s, 120 s). The aim of the present study was to trace the period of seedling formation and to establish the effect of laser irradiation on the duration of the phenological growth stages.

Mathematical and statistical processing methods are applied to the database of experimental data. It was found that pre-sowing laser irradiation of tobacco seeds with a red laser led to an acceleration of germination. The results show that the wide application of pre-sowing laser irradiation of tobacco seeds will enable intense and more qualitative tobacco leaf production, as well as the protection of the environment.

Keywords: laser radiation, tobacco growth, regression analysis

**Introduction**

Tobacco (*Nicotiana tabacum* L.) is an important model plant that is widely used to study genetic and physiological responses in plants. It is still important for the Bulgarian economy. Bulgaria is the only one in the European Union, where three tobacco types are grown – sun-cured (Oriental) - and large-leaf ~~–~~ Flue-cured (Virginia) and Air Cured (Burley).

Traditionally, tobacco seedlings in Bulgaria are grown in ordinary beds in polyethylene tunnels (Atanasov, 1972) or in water beds (floating system) (Dimitrov et al., 2005).

The production of tobacco seedlings in water beds is an alternative way of growing plants, with a number of advantages of seedlings - evenness of seedlings, maintaining the integrity of the root system, quick interception of the field (Dimitrov et al., 2005), lower costs and less labor-intensive (Yang Chunlei et al., 1997), seedling without disease (Kabranova et al., 2014). Seedling production of ordinary beds is more labor-intensive and is influenced by climatic conditions. In this way, seedlings of medium quality are produced (Kabranova et al., 2014).

Shortening the days of germination in Virginia tobacco, accelerating the growth and development of plants, the short growing season are an advantage for Virginia tobacco. Aladjadjian & Ylieva (2003) investigated the effect of a constant magnetic field with an induction of 0.15 T at exposures of 10 min, 20 min and 30 min on tobacco seeds (Nicotiana tabacum L.) to determine the effect on seed viability. They found that the magnetic field stimulates the development of sprouts, leads to increased germination energy and germination. Zhao et al. (1997) studied plant specimens, including tobacco, irradiated with a laser. They found that tobacco seedlings grew very quickly.

There are a few reports in the literature on the effects of laser on various agrobiological indicators in plants (Abbas et al., 2017; Buschmann et al., 2000).

The aim of the present study was to determine the effect of irradiation of Virginia tobacco seeds with red laser on the duration of the phenological stages of growth and to trace the period of seedling formation.

**Materials and Methods**

The study was conducted in 2021 under laboratory and field conditions. The subject of the study were three genotypes of Virginia tobacco - VD, OX 207 and L 6.3.3, whose seeds were irradiated with a red laser (χ= 655 nm, P = 0,7 mW) with different durations (30 s, 60 s, 120 s) before sowing. The field experiment was embedded in the experimental field of the Tobacco and Tobacco Products Institute, Markovo, Bulgaria. Tobacco seedlings are grown in ordinary beds covered with polyethylene canvas.

To study the effect on the duration of seed irradiation within the duration of distribution formation, a one-way analysis of variance was applied using the Fisher test and the LSD test to assess differences with undescribed seeds at a statistical significance level of 0,5. Calculation of the average value of each of the indicators at the corresponding duration of the interruption, the standard deviation, the standard error of average and other statistical values.

Pearson's correlation coefficients are calculated, which determine the direction and strength of the relationship between the duration of irradiation and agrobiological indicators. Statistically proven correlations are a prerequisite for applying regression analysis in order to present them in analytical form. IBM SPSS Software is used for mathematical data processing (Landau & Everitt, 2004).

**Results and discussion**

As a result of the one-factor analysis of variance by Fisher's exact test it was proved that the duration of laser irradiation has a statistically proven positive effect on the duration of all phases of seedling formation from sowing to "seedling emergence age 10%" and "seedling emergence age" 50% '; "4-leaf growth stage (day) 10%" and "4-leaf growth stage (day) 50%"; "5-leaf growth stage (day) 10%", "5-leaf growth stage (day) 50%"; "Mature seedling age (day) 10%" and "Mature seedling age (day) 50%" (Table 1). For each of these indicators, it was found that as the effects of radiation on tobacco seeds increased, these periods decreased (Table 2). Shortening the days of germination in Virginia tobacco, accelerating the growth and development of plants is an advantage in the cultivation of Virginia tobacco seedlings.

In non-irradiated seeds, seedling emergence age of 10% from sowing to germination is reached after 23 days, and after irradiation up to 120 seconds this period decreases to 18 days. Early and rapid growth of tobacco is desirable both to avoid damage from fertilizers (Clarke and Reed, 2001) and to meet the requirements of tobacco growers for early and high quality seedlings. After laser irradiation, the beginning of this phase is shortened by 13%. The duration of irradiation is in a significant, negative relationship with the onset of germination (*r* = -0.611). For plants that are not exposed to laser, the mass germination of seedling emergence age is observed 50% on the 25th day of sowing, and for 30-second irradiated - a shorter period is required – 22,7 days, at 60-second - 20 days, and at 120-second - up to 19,3 days, or over 12% reduction of the period. The relationship with the treatment time is significantly negative (*r* = -0,709 \*\*).

The beginning of the phase 4-leaf growth stage (day) 10% in non-irradiated seeds is reached after 34,7 days, and after irradiation from 27.7 days in 30 seconds, to 26 - in 60 seconds and 25,7 - at 120 seconds. Here, a reduction of the period from sowing to the beginning of the 4-leaf growth stage (day) 10% phase is found by more than 15%. Significant, negative effect of irradiation time (*r* = -0,661 \*) is proven.

In the phase of 4-leaf growth stage (day) 50% this period is shortened again by more than 15%, as in non-irradiated seeds it lasts 35,7 days, and in those subjected to 120-second irradiation – 26,7 days after germination, i.e. decreases by 25%. The relationship between the two studied indicators is significant, negative (*r* = -0,757 \*\*).

The onset of the "5-leaf growth stage (day) 10%" phase in non-irradiated seeds lasts 46 days, in 30-second irradiation – 35,7 days, in 60-second - 34 days, and in 120 – 31,7 days from germination, i.e. the period is shortened at 120 seconds by 31%, and the favorable effect of irradiation is proved by the negative correlation coefficient (*r* = -0,777 \*\*).

The average duration of the period up to 5-leaf growth stage (day) 50% is 38,4 days, as in non-irradiated seeds it is 47,3, and in 120-second irradiation – 33,3, which is a reduction of 29,5% at irradiation 120 seconds and 18,8% - from the average duration of the period. The negative relationship between the irradiation time and the period up to this phase is proved by the value of the correlation coefficient (*r* = -0,791 \*\*).

The beginning of the "Mature seedling age (day) 10%" phase with non-irradiated seeds occurs on the 64th day after sowing, after 30 seconds of irradiation - 62.7 days after sowing, after 60 seconds – 59,3 days, and after 120 seconds - 57 days after sowing. The average duration of this period is 60.8 days, which is 5% shorter than that of non-irradiated seeds and 11% shorter than that of 120-second irradiation (correlation coefficient equal to *r* = -0,829 \*\*).

As the duration of laser exposure decreases, so does the period to maturity seedling age (day) of 50% from 64.7 days in non-irradiated to 60 days in irradiated for 120 seconds, ie. by more than 7% (*r* = -0,735 \*\*). The results show that the growth of Virginia tobacco seedlings is stimulated by laser radiation.

The presence of statistically proven, significant to high correlation coefficients determines the need for regression analysis*.* It provides an opportunity to present the established relationships in an analytical form. The compiled mathematical models are statistically significant at the level of 0,05. The coefficients in front of the independent variable (x - duration of irradiation) in each of them has a negative sign, which again proves the negative relationship between irradiation time and each of the studied agro-biological indicators and the beneficial effect of the laser on plants in terms of seed germination to the "Mature seedling age" phase.

Figure 1 presents the effect of the red laser irradiation (χ = 655 nm, P = 0,7 mW) on the tobacco seeds, from the Virginia variety group, on the length of the periods up to seedling emergence age and 4-leaf growth stage-2 is presented the influence of the duration of red laser irradiation (χ = 655 nm, P = 0,7 mW) on the length of the periods up to seedling emergence age and 4-leaf growth stage.

Figure 2 presents the effect of the red laser irradiation (χ = 655 nm, P = 0,7 mW) on the tobacco seeds, from the Virginia tobacco type on the length of the periods up to 5-leaf growth stage and mature seedling age

The constructed trend lines prove the tendencies of shortening the duration of these periods, as a result of increasing the time of exposure to the laser.

**Conclusions**

Тhe effect of irradiation of Virginia tobacco seeds with red laser on the duration of the phenological stages of growth is investigated for the first time. Mathematical and statistical processing methods are applied to the database of experimental data. The compiled mathematical models are statistically significant at the level of 0,05. All coefficients in front of the independent variable have a negative sign, which proves the negative relationship between irradiation time and each of the studied agro-biological indicators. Thus the beneficial effect of the laser irradiation on Virginia tobacco plants in terms of seed germination to the "Mature seedling age" phase is proven. It was found that pre-sowing laser irradiation of tobacco seeds with a red laser led to an acceleration of germination. The results show that the wide application of pre-sowing laser irradiation of tobacco seeds will enable intense and more qualitative tobacco leaf production, as well as the protection of the environment. The results obtained for the influence of preliminary irradiation of seeds with laser radiation could be of interest to the scientific community in the field of cultivated industrial crops in terms of using laser radiation to stimulate germination and growing of industrial crops of interest.

**Acknowledgements**

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018.

**References**

Abbas, M., Arshad, M., Nisar, N., Nisar, J., Ghaffar, A., Nazir, A., Tahir, A., Iqbal, M. 2017, Muscilage characterization, biochemical and enzymatic activities of laser irradiated Lagenaria siceraria seedlings, Journal of Photochemistry and Photobiology B: Biology, 173, 344-352

Aladjadjian, A., Ylieva, T. 2003, Influence of stationary magnetic field on the early stages of the development of tobacco seed (*Nicotiana tabacum L*.), Journal of Central European Agriculture (online), Volume 4 (2003) No2, pp. 132-137

Atanasov D. 1972. Tobacco Production in Bulgaria. Hr. G. Danov: Plovdiv; 430 pp (in Bulgarian)

Buschmann, C., Langsdorf, G., Lichtenthaler, H. 2000, Imaging of the Blue, Green, and Red Fluorescence Emission of Plants: An Overview, Photosynthetica 38, 483–491. https://doi.org/10.1023/A:1012440903014

Clarke, J.J, Reed, T. D. 2001, Development of a greenhouse tobacco seedling performance index. Tobacco Science, 45: 49–55. https://doi.org/10.3381/0082-4623-45.1.49

Dimitrov, A., Bozukov, H., Nikolov, P., & Drachev, D. (2005), Tobacco production for farmers. Ed. “Videnov and Son”, Sofia. ISBN 954-8319-40-3 (In Bulgarian)

Kabranova, R., Arsov, Z., Dimov, Z. 2014, Marijana Spirkovsk Impact of float tray technology on quality of oriental tobacco seedling, 49th Croatian & 9th International Symposium on Agriculture, Dubrovnik, Croatia, 362-366.

Landau, S., Everitt, B. 2004. A Handbook of Statistical Analyses using SPSS, Chapman & Hall/CRC, London, UK, 137 p.

Yang Chunlei, Hu Yongxiong, Chen Guohua Gao Aifei, Lu Zhengbin,Yang Xinghe, 1997, Study on the Technique of Tobacco Seedling Production with the Direct-seeded Float System. Acta Tabacaria. en.cnki.com.cn

Yang Zhao, Junlan Wang, Xiaoping Wua, Fred W. Williams, Richard J. Schmidt. 1997, Point-wise and Whole-field Laser Speckle Intensity Fluctuation Measurements Applied to Botanical Specimens. Optics and Lasers in Engineering, 28 (6) 443-456.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Duration of laser irradiation | Seedling emergence age, 10% | Seedling emergence age,  50% | 4-leaf growth stage,  10% | 4-leaf growth  Stage,  50% | 5-leaf growth stage,  10% | 5-leaf growth stage, 50% | Mature seedling age,  10% | Mature seedling age, 50% |
| 0 | 23 | 25 | 34,7 | 35,7 | 46 | 47,3 | 64 | 64,7 |
| 30 | 20,7\* | 22,7\* | 27,7\* | 31\* | 35,7\* | 38\* | 62,7\* | 63\* |
| 60 | 18,3\* | 20\* | 26\* | 27,7\* | 34\* | 35\* | 59,3\* | 61\* |
| 120 | 18\* | 19,3\* | 25,7\* | 26,7\* | 31,7\* | 33,3\* | 57\* | 60\* |
| AVERAGE | 20 | 21,8 | 28,5 | 30,3 | 36,8 | 38,4 | 60,8 | 62,2 |
| STD. DEV. | 2,985 | 3,049 | 4,583 | 4,372 | 6,250 | 6,156 | 3,388 | 2,443 |
| SEM. | 1,528 | 0,880 | 1,323 | 1,262 | 1,804 | 1,777 | 0,978 | 0,705 |
| SS | 48,667 | 60,917 | 159,000 | 148,250 | 360,333 | 351,583 | 90,917 | 39,000 |
| F-Test | 2,120 | 3,930 | 5,889 | 6,376 | 13,859 | 14,350 | 6,862 | 3,900 |
| F-crit. | 0,860 | 0,860 | 0,860 | 0,860 | 0,860 | 0,860 | 0,860 | 0,860 |
| Sign. | 0,122 | 0,054 | 0,020 | 0,016 | 0,002 | 0,001 | 0,013 | 0,055 |

Table 1: Results of the study of the effect of laser irradiation (s) on Virginia tobacco seeds on seedling formation (day) by one-way analysis of variance and LSD-test to assess differences at a level of statistical significance of 0,5; AVERAGE - average value; STD. DEV. - standard deviation; SEM - Standard Error of Means; SS - Sum of Squares; F-Test - Fisher’s exact test; F-crit. - F critical value; Sign. - Significance value at Significance Level 0,5

|  |  |  |
| --- | --- | --- |
| Indicators | Correlation coefficients | Regression equations |
| Seedling emergence age, 10% | -0,611\* | *y*=-0,04*x*+22,2 |
| Seedling emergence age, 50% | -0,709\*\* | *y*=-0,04*x*+24,2 |
| 4-leaf growth stage, 10% | -0,661\* | *y*=-0,07*x*+31,93 |
| 4-leaf growth stage, 50% | -0,757\*\* | *y*=-0,07*x*+34 |
| 5-leaf growth stage, 10% | -0,777\*\* | *y*=-0,1*x*+42,3 |
| 5-leaf growth stage, 50% | -0,791\*\* | *y*=-0,11*x*+43,9 |
| Mature seedling age, 10% | -0,829\*\* | *y*=-0,06*x*+63,9 |
| Mature seedling age, 10% | -0,735\*\* | *y*=-0,03*x*+64,2 |

Table 2. Correlation coefficients (*r*) and regression equations, representing the relationship between the duration of irradiation (*x*, day) and the growth phases (*y*)

\* Statistical proof of the coefficient at level 0,05

\*\* Statistical proof of the coefficient at level 0,01

Figure 1. Influence of the duration of red laser irradiation (χ = 655 nm, P = 0,7 mW) on the length of the periods up to seedling emergence age and 4-leaf growth stage

Figure 2. Influence of the duration of red laser irradiation (χ = 655 nm, P = 0,7 mW) on the length of the periods up to 5-leaf growth stage and mature seedling age