



Laser Radiation to Stimulate Tobacco Growth

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ABSTRACT

Background: During the last 50 years the progress of chemical technology provoked a production revolution in 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 agricultural produce and 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.

Methods: The subjects of this study were three genotypes of Virginia tobacco, whose seeds were irradiated with a red laser ($\lambda=655$ nm, $P=0.7$ mW) with different durations (30s, 60s, 120s). The present study aimed 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.

Result: 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.

Key words: Laser stimulation, Phenological growth stages, Red laser, Regression analysis, Seedling formation, Virginia tobacco.

INTRODUCTION

Recently the use of physical methods for plant growth stimulation and for seed germination is getting very popular (Govindaraj *et al.*, 2017; Simonova *et al.*, 2020; Botey *et al.*, 2022). Physical factors can be used to get positive biological changes in crop plants without harmful ecological effects (Ruiz-nieto *et al.*, 2022; Zulkifli *et al.*, 2023).

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 four varieties of tobacco are grown - Oriental - variety Basma and Kaba Kulak and large-leaved - varieties Virginia Flue-cured and Burley- Air cured.

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 *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 (Aladjadjan and Ylieva, 2003) investigated the effect of a constant magnetic field with an induction of 0.15T at exposures

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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.

Recently, laser application in agriculture has gained much attention since plant characteristics were improved significantly in response of pre-sowing seed treatment (Urva *et al.*, 2017). Laser biostimulation of seeds has established itself as a safe and sustainable alternative to genetic modification or chemical use to enhance plant germination and growth. A knowledge gap, however, exists to define optimal laser parameters for different seeds as inappropriate irradiation may seriously damage or destroy germinability (Nadimi *et al.*, 2022).

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; Hassan *et al.*, 2020a, 2020b; Hernandez-aguilar *et al.*, 2006, 2009). Two laser sources operating at wave lengths of 632.8 nm (He-Ne) and 488 nm (Ar+) are used in examining the effects of coherent, low-power, continuous wave exposure on the development of *Vigna radiata* L. For both laser wavelengths, an optimal exposure time of 2 minutes is determined for enhanced growth of mung bean seedlings (Janayon and Guerrer, 2019).

The exposure of seeds to He-Ne laser light improved the germination rate (Podlesna *et al.*, 2015).

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 ($\lambda = 655$ nm, $P = 0.7$ mW) with different durations (30 s, 60 s, 120 s) before sowing.

The field experiment was carried out in the experimental field of the Institute of Tobacco and Tobacco Products, Markovo, Bulgaria. The soil composition of the beds includes: humus-2.94%, total nitrogen - 0.163%, phosphorus - 2.61%/100 g and pH- 8.08%.

Sowing of irradiated and non-irradiated Virginia tobacco seeds was carried out in common beds of 1 m width and height 10-15 cm. in a dry way and the seeds were mixed with clean baked sand in a ratio of 1:200. Immediately after sowing, the bed was evenly covered with a nutrient mixture.

Tobacco seedlings were grown in polythene tunnels. The frequency of watering was dependent on soil moisture following the Instruction for growing tobacco from the "Virginia" varietal group, www.mzh.government.bg.

The experiment was performed in groups of three replicates. 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 and 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,

Table 1: Results of the study of the effect of laser irradiation on Virginia tobacco seeds on seedling formation by one-way analysis of variance and LSD-test to assess differences at a level of statistical significance of 0.5.

Duration of laser irradiation (s)	Seedling emergence age (day), 10%	Seedling emergence age (day), 50%	4-leaf growth stage (day) 10%	4-leaf growth stage (day) 50%	5-leaf growth stage (day) 10%	5-leaf growth stage (day) 50%	Mature seedling age (day) 10%	Mature seedling age (day) 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

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.

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 (-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 (-0.709**).

In non-irradiated seeds, the 4-leaf growth stage (day) 10% occurs 34.7 days after germination, in irradiation of 30 sec-the phase begins 27.7 days after germination, in 60

sec-26 days and in 120 sec- 25 , 5 days after germination. Here, a 10% reduction in the period from sowing to the beginning of the 4-leaf growth stage (day) by more than 15% is found. Significant, negative effect of irradiation time (-0.661*) is proved.

The beginning of the phase "Crossbreeding" 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 "Crossbreeding" phase is found by more than 15%. Significant, negative effect of irradiation time (-0.661*) is proved. 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 (-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

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

Indicators	Correlation coefficients	Regression equations
Seedling emergence age (day), 10%	-0.611*	$y = -0.04x + 22.2$
Seedling emergence age (day), 50%	-0.709**	$y = -0.04x + 24.2$
4- leaf growth stage (day), 10%	-0.661*	$y = -0.07x + 31.93$
4- leaf growth stage (day), 50%	-0.757**	$y = -0.07x + 34$
5- leaf growth stage (day), 10%	-0.777**	$y = -0.1x + 42.3$
5- leaf growth stage (day), 50%	-0.791**	$y = -0.11x + 43.9$
Mature seedling age (day), 10%	-0.829**	$y = -0.06x + 63.9$
Mature seedling age (day), 50%	-0.735**	$y = -0.03x + 64.2$

*Statistical proof of the coefficient at level 0.05.

**Statistical proof of the coefficient at level 0.01.

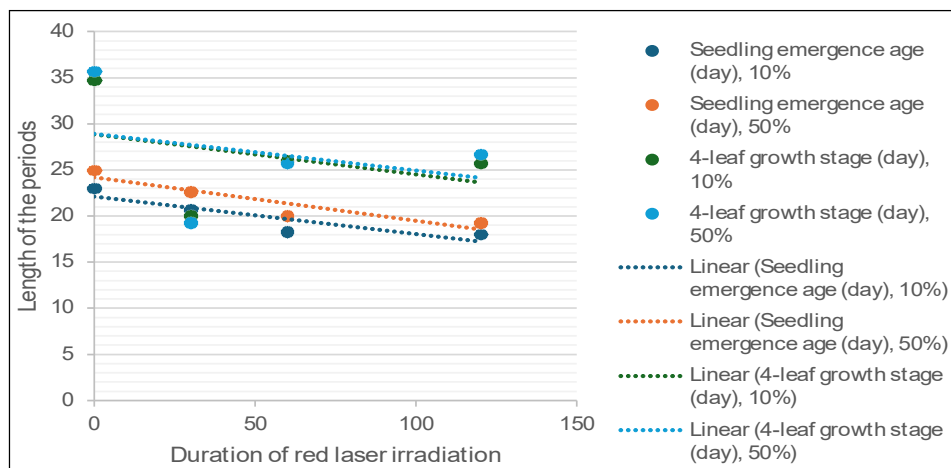


Fig 1: Influence of the duration of red laser irradiation ($c = 655 \text{ nm}$, $P = 0.7 \text{ mW}$) on the length of the periods up to seedling emergence age and 4-leaf growth stage.

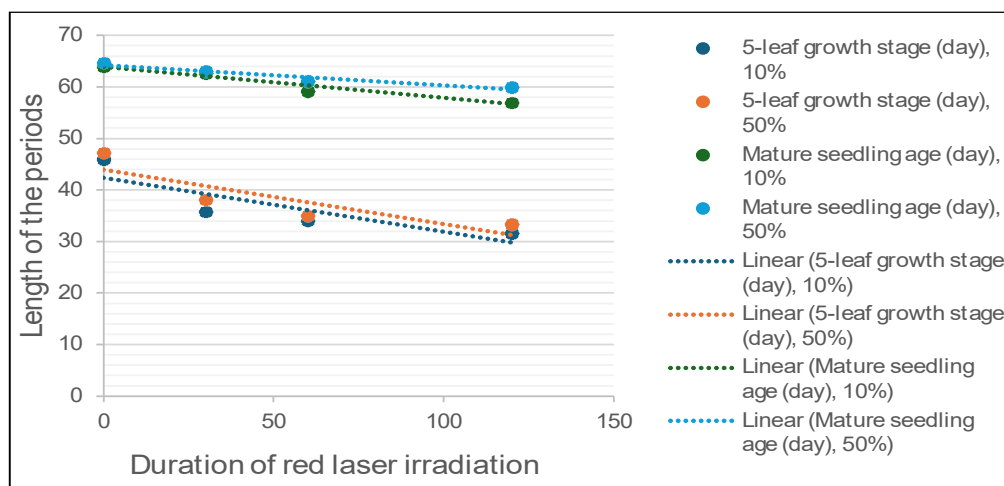


Fig 2: Influence of the duration of red laser irradiation ($c=655\text{ nm}$, $P=0.7\text{ mW}$) on the length of the periods up to 5-leaf growth stage and mature seedling age.

by 31% and the favourable effect of irradiation is proved by the negative correlation coefficient (-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 (-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 -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, i.e. by more than 7% (-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.

Fig 1 presents the effect of the red laser irradiation ($c=655\text{ nm}$, $P=0.7\text{ mW}$) 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 ($c=655\text{ nm}$, $P=0.7\text{ mW}$) on the length of the periods up to seedling emergence age and 4-leaf growth stage.

Fig 2 presents the effect of the red laser irradiation ($c=655\text{ nm}$, $P=0.7\text{ mW}$) on the tobacco seeds, from the Virginia variety group 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.

It was shown that the low-level laser irradiation with He-Ne acts as a bio-stimulator and enhances seed germination of tobacco seeds. A significant enhancement was observed in germination time of laser-irradiated groups than control groups. Seed germination is a complex process regulated by external and internal signals and controlled by plant hormones. Further studies are necessary to understand the exact molecular mechanism involved in the laser bio-stimulation of seed germination.

CONCLUSION

The 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.

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Conflict of interest

I declare here no conflict of interest on behalf of all manuscript authors.

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*. **4(2)**: 132-137.
- Atanasov, D. (1972). Tobacco Production with Drying and Handling. Hristo G. Danov, Plovdiv, p. 200.
- Botey H.M., Ochudho J.O., Ngode L., Dwamena H., Osei-Tutu, I. (2022). Temperature and light effects on germination behaviour of african eggplant (*Solanum aethiopicum* L.) Seeds. *Indian Journal of Agricultural Research*. **2**: 122-128. doi:10.18805/IJARE.A-623.
- 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 green house 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, Sofia, Bulgaria, p. 48.
- Govindaraj, M., Masilamani, P., Alex, Albert, V. and Bhaskaran, M. (2017). Effect of physical seed treatment on yield and quality of crops: A review. *Agricultural Reviews*. **38(1)**: 1-14. Print ISSN: 0253-1496/Online. ISSN: 0976-0539. doi: 10.18805/ag.v0iOF.7304.
- Hasan, M., Hanafiah, M., Taha, Z., Intsar H., Hilfy, Said, M. (2020a). Laser irradiation effects at different wavelengths on phenology and yield components of pretreated maize seed. *Appl. Sci*. **10**: 1189. doi: 10.3390/app10031189. <http://www.mdpi.com/journal/applsci>.
- Hasan, M., Hanafiah, M., Taha, Z., Alhilfy, I. (2020b). Effect of low-intensity laser irradiation on field performance of maize (*Zea mays* L.) emergence, phenological and seed quality characteristics. *Applied Ecology and Environmental Research*. **18(4)**: 6009-6023.
- Hernandez-Aguilar, C., Carballo, C., Artola, A., Michtchenko, A. (2006). Laser irradiation effects on maize seed field performance. *Seed Science and Technology*. **34(1)**: 193-197. <https://doi.org/10.15258/sst.2006.34.1.21>.
- Hernandez-aguilar, C., Dominguez- pacheco, A., Cruz-orea, A., Ivanov, R., Carballo-carballo, A., Zepeda-bautista, R., Galindo Soria, L. (2009). Laser irradiation effects on field performance of maize seed genotypes. *Int. Agrophysics*. **23**: 327-332. [agro.icm.edu.pl](https://doi.org/10.15258/sst.2006.34.1.21).
- Janayon, R., Guerrero, R. (2019). Laser irradiation of mung bean (*Vigna radiata* L.) at two wavelengths for enhanced seedling development. *Hindawi International Journal of Optics* Volume Article ID 3479562, p. 7. <https://doi.org/10.1155/2019/3479562>.
- Kabranova, R., Arsov, Z., Dimov, Z. (2014). Marijana spirkovsk impact of float tray technology on quality of oriental tobacco seedling. 49th Croatian and 9th International Symposium on Agriculture | Dubrovnik | Croatia. pp 362-366.
- Landau, S., Everitt, B. (2004). A Handbook of Statistical Analyses using SPSS, Chapman and Hall/CRC, London, UK, p. 137.
- Nadimi, M., Da-Wen, S., Paliwal, J. (2022). Effect of laser biotimulation on germination of weat. *Applied Engineering in Agriculture*. **38(1)**: 77-84. doi: 10.13031/aea.14772) @2022.
- Podleceña, A., Gładyszewska, B., Podleceńy, J., Zgrajka, W. (2015). Changes in the germination process and growth of pea in effect of laser seed irradiation. *Int. Agrophys*. **29**: 485-492. doi: 10.1515/intag-2015-0054.
- Ruiz-nieto, Jorge, E., Hernández-Ruiz Jesús, Sanzón-Gómez Diana, Isirdia-Lachica Paula C., Zárate-Castrejón José Luis, Mireles-Arriaga Ana I. (2022). Functional lentil sprouts produced under different led light wavelengths Conditions. *Legume Research- An International Journal*. **45(8)**: 1059-1062. doi: 10.18805/LRF-657.
- Simonova, E.N., Ayrapetyan M.M., Eroshenko A.A., Vertiy N.S. (2020). The Impact of UV Irradiation of winter wheat Seeds on enzymatic activity in the germination period. *Indian Journal of Agricultural Research*. **54(2)**: 232-236. doi: 10.18805/IJARE.A-467.
- Urvaa, Shafique, H., Jamil, Y., Haq, Z.U., Mujahid, T., Khan, A.U., Iqbal, M. (2017). Mazhar Abbase Low power continuous wave-laser seed irradiation effect on *Moringa oleifera* germination, seedling growth and biochemical attributes. *Journal of Photochemistry and Photobiology, B: Biology* **170**: 314-323. <https://www.sciencedirect.com/journal/journal-of-photochemistry-and-photobiology-b-biology>.
- Yang, C., Hu Y., Chen, G.G.A., Lu Z., Yang, X. (1997). Study on the technique of tobacco seedling production with the direct-seeded float system. *Acta Tabacaria*. en.cnki.com.cn
- Yang, Z., Junlan, W., Xiaoping, W., Fred, W.W., Richard, J.S. (1997). Point-wise and whole-field laser speckle Intensity fluctuation measurements applied to botanical specimens. *Optics and Lasers in Engineering*. **28(6)**: 443-456.
- Zulkifli, N.A., Khairul Izhar M.A.A., Lau H.Y., Azizi M.M.F. (2023). Advances in Irradiation technology for plant disease resistance: A review. *Agricultural Reviews*. **45(1)**: 25-34. doi: 10.18805/ag.RF-284.