The Journal of Animal & Plant Sciences, 27(3): 2017, Page: 894-900
ISSN: 1018-7081

INFLUENCE OF PRE-SOWING HE-NE AND SEMICONDUCTOR LASER STIMULATION ON SAINFOIN SEEDS GERMINATION FOUR AND EIGHT MONTHS POST-HARVEST

Dragoslav Djokić[1], Milesa Srećković[2], Zoran Fidanovski[3], Jelena Ilić[4], Rade Stanisavljević[5], Dragan Terzić[1], Jordan Marković[1] and Saša Barać[6]

[1]Institute for Forage Crops, 37251 Globoder-Kruševac, Serbia
[2]School of Electrical Engineering, University of Belgrade, Bulevar kralja Aleksandra 73, 11120 Belgrade, Serbia
[3]The School of Computing, Union University, Knez Mihailova 6, 11000 Belgrade, Serbia
[4]Faculty of Mechanical Engineering, University of Belgrade, Kraljice Marije 16, 11120 Belgrade 35, Serbia
[5]Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia
[6]Faculty of Agriculture, University of Priština, Kopaonička bb, 38219 Lešak, Serbia
*Corresponding author's e-mail: dragoslav.djokic@ikbks.com

ABSTRACT

Sainfoin seeds were irradiated by four different lasers (He-Ne laser, three semiconductor lasers of wavelengths 670 nm, 890 nm and of wavelength range 630-680 nm). Each group of seed was irradiated only once, using different exposure times: 1-, 2-, 3- and 4- minutes. The same procedure was applied before autumn (four months post-harvest) and spring sowing (eight months post-harvest). Laser bio-stimulation showed that the optimal operation of the laser was at 890 nm using 3-minutes exposure time in both sowing periods. Slightly weaker, but also significant ($p \geq 0.01$) impact on improving germination was in case of laser of 890 nm using 2- and 4- minutes of exposure time, and 630-680 nm laser using 4- minutes exposure time in both sowing periods. In the spring sowing, only laser of 632.8 nm using 3- minutes exposure time and laser of 670 nm laser between 2- and 4- minutes exposure time had significant improvement of seed germination. In the autumn sowing, He-Ne lasers (632.8 nm) using 4- minutes exposure time, semiconductor lasers of 670 nm laser using 3- minutes exposure time, and 630-680 nm laser using 3- minutes exposure time, also significantly ($p \geq 0.01$) increased seed germination compared to control.

Key words: post-harvest period, sainfoin, seeds, laser stimulation, germination.

INTRODUCTION

Sainfoin (*Onobrychis viciifolia* Scop.) is a perennial forage crop that belongs to the family of legumes. It is cultivated in Central and South Europe, North America, West and Central Asia, although its importance is greater in arid regions (Carbonero *et al*. 2012; Jafari *et al*. 2014). Like its relatives, such as alfalfa, red clover and bird's trefoil, sainfoin is high quality plant regarding both nutritive substances and yield of dry matter. It is especially valued by farmers and geneticists for its ability to recover the soil fertility and for avoiding bloating in animal nutrition.

Sainfoin is planted mixed with forage grasses or as pure crop. For pure crop 100-150 kg ha^{-1} of seeds is required. For the sowing of other forage crops 10-15 kg ha^{-1} of seeds is enough. Thus, seed quantity per unit area is by far greater for sainfoin then for other forage crops. In optimizing quantity of seeds for sowing, percentage of seeds germinating plays a significant role. High seeds germination rate reduces the quantity of seeds required per unit sowing area. However, perennial forage plants usually have low percentage of seeds germinating (Majidi and Barati, 2011). The seeds germination is determined by biological issues (endogenic rhythm, ontogenetic development and dormancy of seeds, microbes, sucking power and the origin of a seed), physical issues (temperature, osmotic pressure, scarification, sunlight and other electromagnetic radiation, ultrasound) and chemical issues (the content of oxygen, water, phyto-hormones, organic acids, pesticides) (Pelemiš *et al.*, 2005). Enhanced seeds germination can be realised by the use of biological treatments (vaccination), genetic modification, chemical and temperature treatments (Kimura and Islam, 2012; Stanisavljević *et al.*, 2012b) or by physical impacts such as damage of seed coat (Dittus and Muir, 2010) and exposure to electromagnetic or magnetic field. Magnetic and electromagnetic pre-sowing treatment have a low environmental impact and at the same time contribute to the increase of yield in crops (Kouchebagh *et al.*, 2014). Magnetic and electromagnetic fields are widely used by many researchers as they fulfil the requirements of organic agriculture (Bilalis *et al.*, 2013).

The aim of this paper was to examine the influence of laser treatment on seeds of sainfoin genotypes in the time of autumn and spring sowing, and to find possibilities to achieve better germinating and seedlings vigour (root and shoot growth and seedling biomass). High percentage of seeds germinating and uniform germination with strong seedling vigour are important prerequisite for successful foundation, fast initial growth and successful agricultural production (Perry, 1980).

MATERIALS AND METHODS

Sainfoin seeds (*Onobrychis viciifolia* Scop.) were collected from three populations in East Serbia and one collection was from sainfoin variety called Macedonian, which has been developed in Republic of Macedonia from local populations. Populations and variety hereafter were referred as genotypes. The seed was subsequently sown at the Institute for forage crops, Kruševac-Globoder (43° 34' N, 21° 12' E and 150 m a.s.l.) in spring of 2010, fully respecting the spatial isolation. Harvesting of seed for further testing was done manually in June 2011. Therefore, the purity was at a high level.

Seeds of all genotypes were dried to moisture content below 12%, after which the mass of 1000 seeds was measured. The effect of genotype was not significant ($p \geq 0.01$) for 1000 seed mass (data not shown), hence the potential effect of seed size on germination and seedling vigour is excluded. Four months after the harvesting (autumn sowing season), and eight months after the harvesting (spring sowing season), seeds were irradiated by four different lasers denoted as *A*, *B*, *C* and *D*. *A* denotes continuous wave (cw) He-Ne laser, wavelength 632.8 nm, output power 1 mW. He-Ne laser, has been chosen because it is one of the most frequently used lasers (Srećković *et al.*, 2010), and the majority of reported laser bio-stimulations had been performed by it. *B* denotes semiconductor laser, wavelength 670 nm, output power 5 mW. It is another red laser, whose wavelength is closer to the wavelength of peak sensitivity of phytochromes, compared to He-Ne laser. *C* denotes low coherent, semiconductor laser, wavelength 630-680 nm, and maximum output power 1 mW. It is low price laser that encompasses broad range of red light. *D* denotes semiconductor laser, wavelength 890 nm, and output power 12 mW. The irradiation by this IR laser shows electromagnetic effects on seeds (non-phytochrome related response).

Seeds were placed in monolayer, taking care to avoid partial screening of seeds. Laser and appropriate lens in front of it were installed in such a way that laser-beam cross-section was expanded into a circular area of 37 mm diameter, that produce light intensities of 0,93 W/m^2 (A and C), 4,65 W/m^2 (B) and 11,16 W/m^2 (D). One side of fixed seeds was irradiated only once. In each irradiation session (autumn and spring), seeds were divided into 17 groups. One was control group that remained not irradiated, but the remaining treatments were exactly the same as in the case of other groups. Four groups were irradiated by laser A with different exposure times: 1, 2, 3 and 4 minutes, and those groups were denoted as *A*1, *A*2, *A*3 and *A*4, respectively. In the same way, another four groups were irradiated by laser *B* and denoted as *B*1, *B*2, *B*3 and *B*4, and so on. In the case of irradiation by each laser type, average intensities (power densities) and doses, are shown in Table 1.

Dose is usually expressed as irradiated energy per unit area. However, bio-stimulated seeds are not uniform surfaces but individual organisms with a complicated heterogeneous composition. Hence, estimating dosage expressing as irradiated energy per seed seems to be worth of consideration (Gładyszewska ~~B.~~, 2011) as well as the question of difference of exposition, absorption and specific dose expressed in this field. In Table 1, values of average doses per unit area (mJ cm^{-2}) and per seed (mJ $seed^{-1}$) are presented.

During autumn and spring sowing, a total of 16 laser treatments were applied on seeds of four genotypes, followed by the examination of their effects in the Seed Laboratory of the Institute for Forage Crops, Kruševac: germination (%), hard seeds (%) and seedling vigour. Seeds were chilled for five days at 5°C. The seed was exposed in the seed germination chambers to alternating temperatures 25/15°C [25°C during 8 h in light (1250 lux) and 15°C during 16 hours in the dark]. Seed germination (%) and hard seeds (%) were determined for each genotype (four replicates of 100 seeds on filter paper) by following ISTA rules (ISTA, 2011).

In addition, seedling vigour was determined in germinated seeds: length of the shoot (cm), length of the root (cm), fresh weight (g) of seedlings (root + shoot) (Stanisavljević *et al*. 2011). Tetrazolium test was applied to separate hard seeds from the dead. The moisture content of composite seed samples was measured thermo-gravimetrically at 130°C to constant weight.

Statistical analysis: F test was used to determine the impact of main factors (genotype and laser type) and their interactions for two periods. LSD test was applied ($p \leq 0.01$) and standard deviation (± SD) to determine the effects of lasers on studied traits. In order to determine the correlation between germination rate and seedling vigour, the simple correlation coefficient (r) was calculated. To correct for non-normality the statistical analysis was done on arcsine transformed values for germination rate and hard seed. For data analysis the SPSS version 11.0 (SPSS inc., USA) and STATISTICA version 8 (StatSoft, Inc. Tulsa, OK, USA) software were used.

RESULTS AND DISCUSSION

During the two studied periods, the effect of genotype and its interaction showed no significant effect ($p \leq 0.05$) on the studied traits (Table 2). Such result was expected, since the genotypes were grown under identical environmental conditions in regard to the applied agriculture practise in crop growing, and the geographical area of genotype origin was small. In such circumstances, genetics did not show a significant effect (Table 2).

Obtained results of uniform seeds germination rate of sainfoin populations and varieties are consistent with the results of other researchers (Stevović *et al.*, 2012). Also, in case of alfalfa, the effect of varieties of the same origin on these features did not show a significant effect (Stanisavljević *et al.*, 2012a). To the contrary, various hybrids showed a significant effect on seed germination and seedling vigour (Hernandez *et al.*, 2010). This is probably because the hybrids were formed from many different genetic parents.

On the other hand the application of the laser resulted in significant ($p \leq 0.01$) increase in germination and seedling vigour, and decrease in occurrence of hard seed in both studied periods (Table 2).

In the agricultural practice in the region of Southeast Europe, sainfoin is the only perennial forage legume where the seed is produced from the first growth, in other words, seed is collected in June (Stevovieć *et al.*, 2012). This allows the seed to mature for about four months before autumn sowing period. Sowing in the autumn compared to sowing in the spring in the next year enables higher yields of forage or seeds in the next year. But in the practice, period of four months is often not sufficient for the seed of perennial forage legumes to increase the germination rate to sufficient level (higher than 75%) to meet legal regulations for market, or to have high seeds germination rate allowing reduction of sowing rates and high seedling vigour. On the other hand, the seedlings from the hard seed occur later in the crop that has already been formed, and cannot compete with classical seed and do not contribute to the desired crop density (Bass *et al.*, 1988).

In autumn sowing period optimal laser treatment (*D*3) had impact on decrease of the occurrence of hard seeds from 9% to 6% and increase of the germination rate from 74% to 79%, growth of shoot for 0.28 cm, growth of root for 0.41 cm, as well as the mass of seedlings for 0.33 g (Table 3).

Seed germination rate in the control group and treatment A1 was statistically significantly lower ($p \leq 0.01$) than in laser treatment in which seed germination rate was 77% or higher. On the other hand, there were significantly more hard seeds in the control group than in groups *B*3, *D*2 and *D*3. Generally, optimal laser treatments had significant positive influence on the seedling vigour (Table 3).

In addition to problem with reduced seed germination rate, deficiency of the autumn sowing period is often lack of or delay of precipitation, which prevents or delays sowing and seedlings are not sufficiently developed before strong frosts.

The spring sowing period starts about eight months after the seed harvest. Compared to the pre-autumn sowing treatments, in the spring period, the germination rate has increased from 76.6% to 82.5%, shoot and root have increased for 0.1 cm each, and mass of seedlings has increased for 0.9 g. Number of hard seeds has decreased from 7.1% to 4.1% (averaged across all genotypes and treatments) (Tables 3 and 4). This can be attributed to the influence of additional maturation of seed.

During the spring sowing period, the optimal laser application (treatment, D3) affected the higher germination rate for 5%, increase in shoot growth for 0.42 cm, increase of small root for 3.36 cm and the mass of seedlings for 1.16 g. Generally, the treatments with higher germination rate and vigour had lower standard deviation (± SD) (Table 4). Apart from D3 treatment, significantly higher ($p \leq 0.01$) seed germination rate was achieved in treatments D2, D4, C3, C4, B3, B4 and A4.

Among other physical methods of germination increase, considerable attention is paid to low intensity laser treatment i.e. laser bio-stimulation (Ćwintal *et al.*, 2010). This method of laser pre-sowing treatment of crop seed may produce the improvement of plant physiological activity and increase in crop yield (Sacała *et al.*, 2012). Nowadays, it is generally considered less harmful to the environment than other methods because it only modifies physiological and natural biochemical processes in seed. In addition, the availability of laser of various wavelengths is growing. Interaction of laser beam and bio-material is the result of various effects such as optical, electromagnetic, thermal etc. In the case of low power lasers, pressure effects can be neglected, and effects of temperature increase is not pronounced (Chen *et al.*, 2005). The laser bio-stimulation method consists of absorbing and storing light energy in plant cells and tissues. Seeds have the same ability as they absorb laser energy, transform it into chemical energy, store it and use it in growth, later. The supply of energy increases the potential power of seeds positively, influencing the intensity of the physiological processes which take place in seeds during germinating (Gładyszewska ~~B.~~, 2011).

The first reaction of seed cells to the incidence of light photons is activation of phytochromes, pigments that are parts of a cell photoreceptor system and regulate growth development of plants. Phytochromes are present in several cell organelles as well as in outer cell membrane – plasmalemma (Hernandez *et al.*, 2010). They are activated when their macromolecules absorb light photons. They are especially sensitive to the absorption of red light photons, and their response shows peak at about 660 nm (Sineshchekov *et al.*, 2000). The activation of phytochromes induces the change of electrochemical potential of cell membrane, which changes the membrane permeability. That change promotes cell metabolism, possibly through the decrease in micro viscosity of intracellular aqueous medium and resultant acceleration of water micro-flows (Samuilov and Garifullina, 2007). Elevated cell metabolism consequently supports the germination process. This process happens when seed is irradiated by common light, such as sunlight, since it also contains the red light, but it is incomparably much more pronounced in the case of seed irradiation by coherent, polarised red light such as laser light. Apart from rapid red light absorption, electromagnetic effects of laser beams also result in energy transfer to a seed that accelerates metabolism and supports germination processes. That is why lasers of, not only red, but also of other wavelengths improve germination and growth (Chen *et al.*, 2010). Additional explanation of positive effects of laser pre-treated seed is the reduction of free radical concentration. This is how the resistance to hostile environment such as drought, chill, humidity is improved (Wu and Zhang, 2007).

The response of the seed to laser irradiation depends on several factors: laser wavelength, duration of a single exposure, repetition of an irradiation, laser intensity, optical properties of seeds and plant variety. Although the reaction to red light is usually more intensive than the reaction to other wavelength, optimum regime of irradiation is still to be found for each plant variety. The influence of pre-sowing laser irradiation on germination and plant growth is particularly thoroughly documented in the case of grains (Perveen *et al.*, 2010, 2011; Sujak *et al.*, 2009; Dziwulska-Hunek *et al.*, 2009). Enhanced germinations were also reported in the case of some vegetables (Muszynski and Gładyszewska, 2008; Muthusamy *et al.*, 2012) and legumes (Podleśny *et al.*, 2012; Ouf *et al.*, 1999). The response of sainfoin seeds to laser irradiation is still unknown.

The correlation between seed germination and seedling vigour: In all cases seeds germination rate showed the positive correlation with seedling vigour traits (Table 5). In general, correlation coefficients showed that the germination rate was in the strongest positive correlation ($p \leq 0.001$) with the vigour of seedlings in the treatments where the germination rate was the highest. These are the treatments D3 and D4 (r = 0.976, r = 0.986, r = 0.979 and r = 0.952 r = 0.946, r = 0.951), respectively, A4 and B4 and C4 for small root and seedling mass (r = 0.926 and r = 0.935, r = 0.925 and 0.936, r = 0.938 and 0.952), A3 for seedling mass (r = 0.936) and D2 treatment for shoot (r = 0.926). On the other hand, the lowest and statistically insignificant correlations between seeds germination rate and seedling vigour traits were established where the seeds germination rate was weak - the control and treatments A1 and A2 for the small root (r = 0.698 r = 0.699) and C1 for shoot (r = 0.699) (Table 4). The positive correlations between seeds germination rate and seedling vigour traits were found in case of Italian ryegrass (*Lolium italicum* A. Braun.), Cocksfoot (*Dactylis glomerata* L.) and timothy (*Phleum pretense* L.) (Stanisavljević *et al.*, 2011).

Therefore, sainfoin seed exposed to optimal laser treatment (which increase germination rate and seedling vigour) and sowed in mixtures with forage grasses with strong seedling vigour could have an impact on improving the cultivation of sainfoin in the mixture. In case of growing in pure culture, exposed sainfoin seed could be better competitor with weeds, growing in the initial growth, which also helps maintain the designed plant density over a period of years. This is one way of controlling other undesirable plant species in the crop.

Table 1. Irradiation intensities and average doses per unit area and per seed (laser type *A* and *C* have the same power).

Laser type	***A,C***				***B***				***D***			
Intensity	0.093				0.465				1.116			
Exposition time	1min.	2min.	3min.	4min.	1min.	2min.	3min.	4min.	1min.	2min.	3min.	4min.
Dose per unit area (mJ cm^{-2})	5.58	11.2	16.7	22.3	27.9	55.8	83.7	112	67.0	134	201	268
Dose per seed (mJ $seed^{-1}$)	1.33	2.66	3.99	5.32	6.65	13.3	20.0	26.6	16.0	31.9	47.9	63.9

Table 2. *F* test for effect of laser and genotype on germination, hard seed and seedling vigour in time of application (A- autumn sowing period and S- spring sowing period).

Source	Time of application	df	Germination %	Hard seed %	Seedling vigour		
					Shoot cm	Root cm	Seedling biomass g.
Laser (L)	**A**	15	**	**	*	*	*
	S	3	*	**	*	*	*
Genotypes (G)	**A**	15	NS	NS	NS	NS	NS

	S	3	NS	NS	NS	NS	NS
LxG	**A**	45	NS	NS	NS	NS	NS
	S	45	NS	NS	NS	NS	NS

Statistical significance level: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, NS not significant

Table 3. Application of laser on sainfoin seed four months post-harvest.

Laser treatment		**Germination %**	**Hard seed %**	**Seedling vigour**		
				Shoot Cm	**Root cm**	**Seedling biomass g.**
Control		74±(8.36) b	9±(3.76) a	2.01±(3.38) b	3.18±(4.03) b	5.78±(5.18) b
A	1 min.-A1	74±(7.96) b	8±(3.36) ab	2.09±(3.03) b	3.23±(4.01) b	5.84±(5.06) b
	2 min.-A2	75±(7.55) ab	7±(3.28) ab	2.19±(3.01) ab	3.24±(3.96) b	5.83±(5.16) b
	3 min.-A3	78±(7.12) a	7±(2.09) ab	2.24±(2.21) a	3.51±(3.16) a	6.09±(4.09) a
	4 min.-A4	77±(7.56) a	6±(2.89) b	2.22±(2.68) a	3.45±(3.29) a	5.91±(4.16) ab
B	1 min.-B1	76±(8.12) ab	8±(3.01) ab	2.19±(2.87) ab	3.27±(3.04) ab	5.89±(5.03) ab
	2 min.-B2	78±(7.05) a	7±(2.09) ab	2.28±(2.09) a	3.52±(2.96) a	6.01±(3.99) a
	3 min.-B3	77±(7.63) a	6±(2.23) b	2.21±(3.08) ab	3.42±(3.01) ab	6.00±(4.09) a
	4 min.-B4	78±(7.13) a	7±(2.19) ab	2.29±(2.14) a	3.49±(2.88) a	6.06±(3.96) a
C	1 min.-C1	74±(8.21) b	8±(3.39) ab	2.08±(3.26) b	3.19±(4.01) ab	5.80±(5.02) b
	2 min.-C2	75±(8.28) ab	7±(3.04) ab	2.12±(3.03) ab	3.25±(3.74) ab	5.83±(4.89) b
	3 min.-C3	77±(7.45) a	7±(2.56) ab	2.23±(2.12) a	3.50±(3.06) a	5.99±(3.99) ab
	4 min.-C4	78±(6.98) a	7±(2.03) ab	2.35±(2.04) a	3.49±(2.88) a	6.08±(3.76) a
D	1 min.-D1	77±(7.76) a	7±(2.85) ab	2.30±(1.96) a	3.51±(3.11) a	5.99±(3.88) ab
	2 min.-D2	78±(7.01) a	6±(2.14) b	2.32±(2.01) a	3.55±(2.78) a	6.08±(3.78) a
	3 min.-D3	79±(6.87) a	6±(2.01) b	2.35±(1.98) a	3.59±(2.87) a	6.11±(3.69) a
	4 min.-D4	78±(7.08) a	7±(2.17) ab	2.29±(2.06) a	3.56±(2.99) a	6.08±(3.83) a
Average		**76.6**	**7.1**	**2.2**	**3.4**	**6.0**

LSD test, Statistical significance level: p ≤0.01. ± standard deviation.

Table 4. Application of laser on sainfoin seed eight months post-harvest.

Laser Treatment		**Seedling vigour**				
		Germination %	**Hard seed %**	**Shoot Cm**	**Root cm**	**Seedling biomass g.**
Control		80±(7.16) b	5±(3.44) a	2.10±(2.99) b	3.25±(3.09) b	6.32±(4.46) b
A	1 min.-A1	80±(6.85) b	5±(3.85) a	2.13±(2.96) b	3.31±(3.04) b	6.66±(4.36) b
	2 min.-A2	82±(5.96) ab	4±(3.33) ab	2.2±(2.78) b	3.40±(3.00) ab	6.86±(3.99) ab
	3 min.-A3	82±(5.88) ab	4±(3.18) ab	2.24±(2.55) ab	3.38±(3.03) ab	6.88±(3.96) ab
	4 min.-A4	84±(5.06) a	3±(2.88) b	2.34±(2.02) ab	3.65±(2.16) a	7.30±(3.35) a
B	1 min.-B1	81±(7.06) ab	5±(4.01) a	2.14±(2.74) b	3.31±(3.07) b	6.35±(4.26) b
	2 min.-B2	82±(7.01) ab	4±(3.12) ab	2.39±(2.48) ab	3.37±(3.02) ab	6.59±(4.01) ab
	3 min.-B3	83±(5.36) a	5±(3.45) a	2.52±(2.06) a	3.58±(2.76) a	6.98±(3.66) a
	4 min.-B4	83±(5.11) a	5±(3.05) a	2.49±(2.05) a	3.56±(2.66) a	6.95±(3.72) a
C	1 min.-C1	82±(6.99) ab	5±(3.86) a	2.49±(2.33) a	3.49±(2.99) ab	6.56±(4.02) ab
	2 min.-C2	82±(6.93) ab	5±(3.04) a	2.48±(2.16) ab	3.41±(2.82) ab	6.49±(3.99) ab
	3 min.-C3	84±(5.02) a	3±(2.36) b	2.52±(1.81) a	3.60±(2.36) a	7.29±(3.39) a
	4 min.-C4	83±(5.66) a	4±(3.01) ab	2.51±(1.89) a	3.54±(3.88) a	7.14±(3.88) a
D	1 min.-D1	82±(6.76) ab	4±(3.56) ab	2.13±(2.87) b	3.39±(3.11) ab	6.98±(4.18) ab
	2 min.-D2	84±(5.01) a	4±(3.26) ab	2.49±(2.00) a	3.56±(3.01) a	7.23±(3.66) a
	3 min.-D3	85±(5.00) a	2±(2.26) b	2.52±(1.56) a	3.61±(2.22) a	7.48±(3.49) a
	4 min.-D4	84±(5.46) a	3±(2.06) b	2.50±(2.01) a	3.59±(2.51) a	7.32±(3.71) a
Average		**82.5**	**4.1**	**2.3**	**3.5**	**6.9**

LSD test, statistical significance level: p ≤0.01. ± standard deviation

Table 5. Correlation coefficients (r) between germination and seedling vigour traits (n=8)

Laser treatments		Seedling vigour		
		Shoot cm	Root cm	Seedling biomass g.
Control		0.658 NS	0.698 NS	0.673 NS
A	1 min.-A1	0.789*	0.698 NS	0.711*
	2 min.-A2	0.778*	0.699 NS	0.709*
	3 min.-A3	0.839**	0.911**	0.936***
	4 min.-A4	0.915**	0.926***	0.935***
B	1 min.-B1	0.785*	0.811*	0.799*
	2 min.-B2	0.819**	0.839**	0.825*
	3 min.-B3	0.831*	0.799*	0.809*
	4 min.-B4	0.913**	0.925***	0.936***
C	1 min.-C1	0.699 NS	0.756*	0.712*
	2 min.-C2	0.789*	0.812*	0.832*
	3 min.-C3	0.801*	0.836**	0.837**
	4 min.-C4	0.919**	0.938***	0.952***
D	1 min.-D1	0.826**	0.799**	0.838**
	2 min.-D2	0.926***	0.915**	0.833*
	3 min.-D3	0.976***	0.986***	0.979***
	4 min.-D4	0.952***	0.946***	0.951***

Statistical significance level: * $P \leq 0.05$, ** $P \leq 0.01$, *** $p \leq 0.001$. NS not significant

Conclusions: Seed treatment with semiconductor laser (890 nm) and exposure of three minutes proved to be optimal for increasing sainfoin seed germination rate before autumn and spring sowing. The treatments with the highest germination rate also showed the strongest positive correlation between germination rate and seedling vigour. Such use of the laser is technically feasible and could be one of the solutions to increase seed germination rate and establishment of more efficient sainfoin crops.

Acknowledgments: Research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia, Project: TR-31057 (2011-2016).

REFERENCES

Bass L.N., R.C., Gunn, B.O. Hesterman and E.E. Roos, (1988). Seed physiology, seedling performance and seed sprouting. in: Alfalfa and alfalfa improvement. Hanson, A.A., K.D., Barnes, R.R., Hill, Jr., eds. ASA, CSSA, and SSSA, Madison, WI. 961-983.

Bilalis J.D., N. Katsenios, A. Efthimiadou, A. Karkanis, .E.M. Khah and T. Mitsis. (2013). Magnetic field pre-sowing treatment as an organic friendly technique to promote plant growth and chemical elements accumulation in early stages of cotton. Australian J. of Crop. Science., 7(1): 46-50.

Carbonero H.C., F. Carbonero, L.M.J. Smith and T.A. Brown (2012). Phylogenetic characterisation of Onobrychis species with special focus on the forage crop *Onobrychis viciifolia* Scop. Genet. Resour. Crop. Evol., 59, 1777-1788.

Chen Y.P., J. Y. Liu, X.L. Wang, Y.Z. Ren and M. Yue (2005). Effect of Microwave and He-Ne Laser on Enzyme Activity and Biophoton Emission of *Isatis indigotica* Fort. J. Integ. Plant Biol. 47(7): 849-855.

Chen Y.P., F. J. Jia and M. Yue (2010). Effect of CO_2 laser radiation on physiological tolerance of wheat seedlings exposed to chilling stress. Photochem. Photobiol., 8: 600-605.

Ćwintal M., P. Sowa and S. Goliasz (2010). Wpływ mikroelementów (B, Mo) i stymulacji laserowej na wartość siewną nasion koniczyny czerwonej. Acta Agroph., 15: 65-76.

Dittus, D.A. and J.M Muir (2010). Breaking germination dormancy of Texas native perennial herbaceous legumes. Native Plan., 11: 5-10.

Dziwulska-Hunek A., K. Kornarzyński, A. Matwijczuk, S. Pietruszewski and B. Szot. (2009). Effect of laser and variable magnetic field simulation on amaranth seeds germination. Int. Agrophysics, 23, 229-235.

Gładyszewska B. (2011). Estimation of a laser biostimulation dose. Int. Agrophysics, 25, 403-405.

Hernandez A.C., A.P. Dominguez, A.O. Cruz, R. Ivanov, A.C. Carballo and R.B. Zepeda (2010). Laser in agriculture. Int. Agrophysics, 24, 407-422.

ISTA (2011) International Rules for Seed Testing. International. Seed Testing Association, Bassersdorf, Switzerland.

Jafari A.A., M. Rasoli, R.S. Tabaei-Aghdaei and P. Salehi (2014). Evaluation of herbage yield, agronomic traits and powdery mildew disease in 35 populations of sainfoin (*Onobrychis sativa*) across 5 environments of Iran. Rom. Agric. Rese., 29: 41-48.

Kimura E., and M.A. Islam (2012). Seed Scarification Methods and their Use in Forage Legumes. Res. J. of Seed Sci., 5: 38-50.

Kouchebagh S.B., F. Farahvash, B. Mirshekari, K. H. Arbat and R. F. Khoei (2014). Seed priming techniques may improve grain and oil yields of sunflower (*Helianthus annuus* L.).The J. Anim. and Plant Sci.,24, 1863-1868.

Majidi, M.M. and M. Barati. (2011). Methods for breaking seed dormancy in one cultivated and two wild *Onobrychis species*. Seed Sci. and Technol., 39(1): 44-53.

Muszyński S. and B. Gładyszewska (2008). Representation of He-Ne irradiation effects on radish seeds with selected germination indices. Int. Agrophysics, 22: 151-157.

Muthusamy A., P.P. Kudwa, V. Prabhu, K.K. Mahato, S.V. Babu, R.M. Rao, M.P. Gopinath and K. Satyamoorthy (2012). Influence of helium-neon laser irradiation on seed germination in vitro and physico-biochemical characters in seedlings of brinjal (*Solanum melongena* L.) var. Mattu Gulla. Photochem. Photobiol., 88(5): 1227-1235.

Ouf S.A. and F.N. Abdel-Hady (1999). Influence of He-Ne laser irradiation of soybean seeds on seed mycoflora, growth, nodulation and resistance to *Fusarium solani*. Folia Microbiol., 44(4): 388-396.

Pelemiš S., S. Babić, R. Vasić, M. Dukić, V. Vujošević-Simić, U. Mioč and Z. Nedić (2005). Uticaj lasera na biljke. Proc. 49th ETRAN conference, Budva, June 5-10, III, 347-350.

Perry D.A. (1980). The concept of seed vigor and its relevance to seed production techniques. In: Hebblathwaite P D, ed., Seed Production. Butterworths, London. Pp. 585-591.

Podleśny J., A. Stochmal, A. Podleśna and E.L. Misiak. (2012). Effect of laser light treatment on some biochemical and physiological processes in seeds and seedlings of white lupine and faba bean. Plant Growth Regul., 67: 227-233.

Perveen R., Q. Ali, M. Ashraf, F. Al-Qurainy, Y. Jamil and R.M. Ahmad. (2010). Effects of different doses of low power continuous wave he-ne laser radiation on some seed thermodynamic and germination parameters, and potential enzymes involved in seed germination of sunflower (*Helianthus annuus* L.). Photoc. Photobiol., 86(5), 1050-1055.

Perveen R., Y. Jamil, M. Ashraf, Q. Ali, M. Iqbal and R.M. Ahmad (2011). Ne-Ne laser-induced improvement in biochemical, physiological, growth and yield characteristics in sunflower (*Helianthus annuus* L.). Photoc. Photobiol., 87(6): 1453-1463.

Sacała E., A. Demczuk, E. Grzyś, U. Prośba-Białczyk and H. Szajsner (2012). Impact of presowing laser irradiation of seeds on sugar beet properties. Int. Agrophysics, 26: 295-300.

Samuilov F.D. and L.R. Garifullina (2007). Effect of Laser Irradiation on Microviscosity of Aqueous Medium in Imbibing Maize Seeds as Studied with a Spin Probe Method. Russian J. Plant Physiol., 54: 128-131.

Sineshchekov V., O. Ogorodnikova, A. Thiele and C. Gatz (2000). Fluorescence and photochemical characterization of phytochromes A and B in transgenic potato expressing *Arabidopsis* phytochrome B. Photochem. Photobiol. Biology, 59: 139-146.

Srećković M., P. Osmokrović, Lj. Konstatinović and V. Arsoski (2010). Izabrane primene lasera u medicini – interakcija lasera sa biomaterijalom, Zavod za fiziku tehničkih fakulteta, Beograd, Serbia.

Stanisavljević R., D. Đokić, J. Milenković, L. Đukanović, V. Stevović, A. Simić and D. Dodig. (2011). Seed germination and seedling vigour of Italian ryegrass, Cocksfoot and Timothy following harvest and storage. Cienc. Agrotec., 35: 1141-1148.

Stanisavljević R., S. Vucković, A. Simić, J. Marković, Z. Lakić, D. Terzić and D. Đokić. (2012a). Acid and Temperature Treatments Result in Increased Germination of Seeds of Three Fescue Species. Not. Bot. Horti. Agrobo., 40: 220-226.

Stanisavljević R., D. Beković, D. Djukić, V.Stevović, D.Terzić, J. Milenković and D. Djokić. (2012b). Influence of plant density on yield components, yield and quality of seed and forage yields of alfalfa varieties. Rom. Agric. Rese., 29, 245-254.

Stevović V., R. Stanisavljević, D. Djukić, D. Djurović. (2012). Effect of row spacing on seed and forage yield in sainfoin (*Onobrychis viciifolia* Scop.) cultivars. Turk. J. Agric. For., 36, 35-44.

Sujak A., A. Dziwulska-Hunek and K. Kornarzyńsky (2009). Compositional and nutritional values of amaranth seeds after pre-sowing He-Ne laser light and alternating magnetic field treatment. Int. Agrophysics, 23, 81-86.

Wu J., X. Gao and S. Zhang (2007). Effect of laser pretreatment on germination and membrane lipid peroxidation of Chinese pine seeds under drought stress. Front. Biol. China, 2, 314-317.