Int. Agrophysics, 2004, 18, 289-293

INTERNATIONAL
Agrophysics
www.ipan.lublin.pl/int-agrophysics

Germination capacity and the health status of red clover seeds following laser treatment**

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Received February 3, 2004; accepted May 20, 2004

A b s t r a c t. Laboratory experiments on the germination of tetraploid red clover seeds (var. Bona) were carried out completely randomly in four replications. The number of seeds germinating normally and abnormally, as well as the number of hard seeds and seeds infected with fungal disease was also determined in the experiment. Laser treatment significantly decreased the share of hard seeds and did not influence the percentage of seeds germinating normally. Seed dressings significantly decreased seed infection with disease when compared to the control and objects with laser treatment. Clover seeds were most abundantly infected by fungi of the *Alternaria* type (*Alternaria alternata*). Strains of the *Phoma* and *Penicillium* type were eliminated by laser beam with power of 3 mW cm^{-2} x 1 and 3 mW cm^{-2} x 3, and *Penicillium* by a dose of 6 mW cm^{-2} x 1 i 6 mW cm^{-2} x 3. Laser treatment should not be applied in the case of massive seed infection with fungi of the *Alternaria* type since a significant increase was noted after laser irradiation with power of 3 mW cm^{-2} x 3; 3 mW cm^{-2} x 5 and 6 mW cm^{-2} x 5.

K e y w o r d s: laser treatment, red clover, germination

INTRODUCTION

The influence of laser light on the germination and stimulation of seeds has been determined in earlier studies (Dziamba and Koper, 1992; Podleśny, 1997; Podleśny, 2002; Szyrmer and Klimont, 1999). Most often, this treatment has resulted in an increase in the ability of seeds to germinate (Podleśny, 1997; Podleśny, 2002). The above mentioned studies concerned mainly annuals, the characteristics of the share of hard seeds and seeds germinating abnormally not always having been given in them.

In the present study, the object was red clover seed (*Trifolium pratense* L.) which is a perennial. In the Polish literature on the subject, no results are available from studies conducted on the laser stimulation of seeds from perennial papilionaceous plants. This fact prompted the present authors to undertake studies to determine the influence of the laser treatment on seeds and the abilities of seeds to germinate, and the share of hard seeds and seeds infected with disease.

MATERIAL AND METHODS

In 2002, during laboratory studies, the following factors were taken into consideration:

1) Irradiation with a divergent He-Ne laser beam with a surface power density in the irradiation plane of 0, 3 and 6 mW cm^{-2}. The seeds were subjected to 1-3 and 5 irradiation rounds on a laser device designed according to the Koper and Dygała patent (1994). A single irradiation round lasted for 0.1 s.

2) Three seed dressings: Funaben T, Sarfun T 65 DS and Super-Homai 70 DS, all taking place in a controlled environment.

Seeds of the new Bona tetraploid variety from the 2001 harvest were used in the present experiment. Germination of the seeds was carried out in accordance with ISTA instructions (Filipowicz and Wagner, 1987) on Petri dishes. Tissue paper soaked with water served as the substrate. One hundred seeds were tested on one dish. Germination took place at a temperature of about 20°C, and the number of seeds which germinated was counted after 4 and 10 days. The number of seeds which germinated normally and

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**The present study was carried out within the framework of research project No 6 P06R 0351 financed by the State Committee for Scientific Research.

abnormally, as well as the number of hard seeds and seeds infected with disease was determined. Normally germinating seeds have a well developed root system with the main root, undamaged parts above and below dicotyledones and two dicotyledones. Abnormally germinating seed do not show properties that would ensure further normal plant growth due to damage and deformations of seedlings present as well as other abnormalities (Duczmal and Tucholska, 2000). The experiment was carried out completely randomly in four replications. In order to identify the fungal strains infecting the alfalfa, appropriate mycological tests were carried out. Seeds with mould were transferred onto a dish with Martin's substrate (Litwinow, 1967) and incubated at 26°C for several days. The fungal mycelium was then separated and transferred onto a substratum with glycosil-potato substrate (PDA). After pure cultures had been isolated, macroscopic and microscopic observations were carried out to determine the species and types of fungi. The final classification of the isolates was done based on their micro- and macro-morphological features in accordance with the studies by Domsch (1980) and Litwinow (1967).

The number of those fungal strains most frequently occurring and the total number of fungi isolated is given in the present study.

The results obtained were then statistically processed using variance analysis and $LSD_{0.05}$ further to Tukey's test.

RESULTS AND DISCUSSION

Red clover of the variety Bona is characterized a high germination ability. Seeds germinating normally constituted about 81% and were not significantly influenced by the factors studied (Table 1). In contrast, the share of seeds germinating abnormally increased significantly under the influence of a higher dose of laser light both at the 3 and 5-fold irradiations. However, the highest increase in the share of seeds germinating abnormally took place under the influence of the Funaben T and Super-Homai 70 DS seed dressings. In all, seed dressings significantly increased the percentage of seeds germinating abnormally when compared to the control and to the objects with laser stimulation of seeds. The total of seeds germinating normally and abnormally was about 90% and did not show any significant variations.

In the control object and the object with the Funaben T dressing, hard seed exceeded 10% (Table 2). On the other hand, the lowest amount of hard seeds was noted when 5- and 3-fold laser irradiations at a dose of 3 mW cm^{-2} of surface power density in the irradiation plane were applied. Similarly, the Super-Homai 70 DS seed dressing, as one of the dressings applied, contributed to the lowering of the percentage share of hard seeds. Considering the share of clover seeds infected with fungal diseases, it should be said that only the seed dressings explicitly decreased their share.

T a b l e 1. The influence of laser treatment and the dressing of red clover seeds on germination

Factors	% of seeds		Total of seeds germinating (%)
	germinating normally	germinating abnormally	
Irradiation dose and multiplication:			
R 0	80.7	7.0	87.7
R 3x1	84.0	6.5	90.5
R 3x3	80.0	11.6	91.6
R 3x5	84.0	4.5	88.5
Mean	82.7	7.5	90.2
R 6x1	83.0	6.0	89.0
R 6x3	80.0	8.0	88.0
R 6x5	81.0	8.5	89.5
Mean	81.3	7.5	88.8
Seed dressings:			
Funaben T	74.0	15.2	89.2
Sarfun T 65 DS	81.0	9.0	90.0
Super-Homai 70 DS	83.0	10.7	93.7
Mean	79.3	11.6	90.9
$LSD_{0.05}$	–	0.81	–

T a b l e 2. The influence of laser treatment and the dressing of red clover seeds on the share of hard seeds and seeds infected with disease

Factors	% of seeds		Total of non-germinating seeds (%)
	hard	infected with diseases	
Irradiation dose and multiplication:			
R 0	10.1	2.2	12.3
R 3x1	7.2	2.3	9.5
R 3x3	6.7	1.7	8.4
R 3x5	6.5	5.0	11.5
Mean	6.8	3.0	9.8
R 6x1	6.0	5.0	11.0
R 6x3	9.7	2.3	12.0
R 6x5	6.8	3.7	10.5
Mean	7.5	3.7	11.2
Seed dressings:			
Funaben T	10.2	0.6	10.8
Sarfun T 65 DS	9.7	0.3	10.0
Super-Homai 70 DS	6.0	0.3	6.3
Mean	8.6	0.4	9.0
$LSD_{0.05}$	0.78	0.21	0.93

Of the group of the seed dressings tested, Funaben T was the least effective. Laser irradiation increased the percentage share of the infected seeds as compared to the control in most cases. The highest (5%) infection with fungal diseases was noted when a 5 fold dose of 3 mW cm^{-2} and a single dose of 6 mW cm^{-2} was applied. The above quoted data proves that the energy of the laser light stimulated development in some fungal strains; hence seeds massively infected by fungal disease should not be subjected to irradiation.

Phyto-pathogenic fungi of the *Alternaria* and *Phoma* types and saprophytic fungi of the *Penicillium* type were the most frequently occurring strains of red clover seeds (Table 3). Seed dressings destroyed fungi of the *Phoma* and *Penicillium* type and limited the *Alternaria* strain to 20% in comparison with the control. An interesting observation is that fungi of the *Phoma* and *Penicillium* types were also completely liquidated by the divergent laser light beam with a power of 3 mW cm^{-2} at 1- and 3-times irradiations. The *Penicillium* strains occurring in the seeds were also totally eliminated at double the power of the divergent beam (6 mW cm^{-2}) when applied singly and 3-fold. Unfortunately, the most frequently occurring strain of *Alternaria* increased under the influence of laser light when a power of 3 mW cm^{-2} was applied 3- and 5-times and when a power of 6 mW cm^{-2} was applied 5-times as compared to the control. Generally, the lowest number of strains isolated (1) was noted in those objects in which seeds were dressed with Sarfun T 65 DS and Super-Homai 70 DS, whereas the highest (22) occurred at a 5-fold seed irradiation with a dose of 3 mW cm^{-2} of surface power density of the divergent laser beam in the irradiation plane.

Table 4 summarizes the influence of the increases in laser irradiation on the germination and health status of red clover seeds irrespective of the divergent laser beam power. The percentage share of seeds germinating normally was similar for the 1- 3- and 5-fold irradiations. The lowest amount of hard seeds and seeds germinating abnormally was noted at the 1- and 5-fold irradiations. The highest amount of seeds infected with fungal diseases was noted at the 5- and 3-fold irradiations. The share of fungi of the *Alternaria* type increased almost proportionally with the 1- to 5-fold irradiations. The remaining strains reacted more weakly to the laser light since only the 5-fold irradiation resulted in any increased activity. Moreover, the 5-fold seed irradiation increased the total number of fungal strains isolated on the seeds of the Bona variety of tetraploid red clover. The types of fungus isolated belonged to the groups of field and repository fungi (Duczmal and Tucholska, 2000; Narkiewicz-Jodko, 1986). Their presence resulted in a decrease of the seed ability to germinate. The dominant groups of clover seeds, potentially, were the phytopathogenic fungi of the *Alternaria* type, *ie Alternaria alternata* and of the

T a b l e 3. The influence of laser treatment and seed dressings on the number of fungi isolated from red clover seeds

Factors	Number of the most frequently occurring strains			Number of strains isolated
	Alternaria	*Phoma*	*Phenicillinum*	
Irradiation dose and multiplication:				
R 0	5.0	2.0	1.0	8.0
R 3x1	2.0	0.0	0.0	5.0
R 3x3	7.0		0.0	8.0
R 3x5	8.0	0.0	1.0	22.0
		5.0		
Mean	5.7	1.7	0.3	11.7
R 6x1	5.0	3.0	0.0	16.0
R 6x3	5.0	1.0	0.0	8.0
R 6x5	11.0	2.0	1.0	16.0
Mean	7.0	2.0	0.3	13.3
Seed dressings:				
Funaben T	1.0	0.0	0.0	6.0
Sarfun T 65 DS	1.0	0.0	0.0	1.0
Super-Homai 70 DS	1.0	0.0	0.0	1.0
Mean	1.0	0.0	0.0	2.7
$LSD_{0.05}$	0.42	0.11	–	0.84

T a b l e 4. The influence of increased laser light irradiation on the germination and health status of red clover seeds

Specification	Multiplication of irradiation			Mean
	1	3	5	
Percentage of seeds:				
germinating normally	83.5	80.0	82.5	82.0
germinating abnormally	6.2	9.8	6.5	7.5
hard	6.6	8.2	6.6	7.1
infected with disease	3.6	2.0	4.3	3.3
Number of the most frequently occurring strains:				
Alternaria	3.5	6.0	9.5	6.3
Phoma	1.5	0.5	3.5	1.8
Penicillium	0.0	0.0	1.0	0.3
Total number of strains isolated	10.5	8.0	19.0	12.5

Phoma - *Phoma medicaginis* var. *pinodella* strain. These latter fungi are classed as toxigenic since they produce metabolites which are toxic both for plants and warm-blooded animals (Filipowicz and Wagner, 1987; Narkiewicz-Jodko, 1986). It should be mentioned here that the above results are original with reference to the problem discussed in this study. Since biodiversity in perennial leguminous plant seeds is very high, studies of this type should be continued to allow us to learn in more detail about the laser influence on hard seeds and seeds infected with fungi, especially of *Alternaria* type (Duczmal and Tucholska, 2000).

CONCLUSIONS

1. The percentage of triploid red clover seeds germinating normally as well as the total of seeds germinating normally and abnormally did not differ significantly under the influence of laser treatment or the application of seed dressings. The percentage share of seeds germinating abnormally increased significantly in those objects where seed dressings were applied.

2. The percentage share of hard seeds decreased significantly under the influence of divergent laser beam power doses *ie* 3 and 6 mW cm^{-2} applied in the irradiation plane and all increases in irradiation.

3. The influence of laser treatment on the percentage of seeds infected with fungal diseases was unclear. A significant increase in seed infection was noted at the following irradiation doses and multiplications: 3 mW cm^{-2} x 5; 6 mW cm^{-2} x 1 and 6 mW cm^{-2} x 5.

4. The application of dressings significantly decreased the share of seeds infected with disease when compared to the control and the objects subjected to laser treatment. Sarfun T 65 DS and Super-Homai 70 DS dressings were characterized with a stronger anti-fungal activity than was the case with the Funaben T.

5. Fungi of the *Alternaria* (*Alternaria alternata*) type had the highest share in the seed inhabitation. The *Phoma* and *Penicillium* strains were eliminated by laser irradiation using a divergent beam in the irradiation plane of 3 mW cm^{-2} x 1 and 3 mW cm^{-2} x 3.

6. Laser treatment of seed should not be applied where it has been infected with the *Alternaria* fungus strain.

REFERENCES

Domsch K.H., 1980. Compendium of Soil Fungi. Academic Press, London.

Duczmal K.W. and Tucholska H., 2000. Seed Science. 1, State Agricultural and Forestry Publications (in Polish). Warsaw.

Dziamba S. and Koper R., 1992. Influence of laser irradiation on the spring wheat yield (in Polish). Fragm. Agron., 1(33), 88-93.

Filipowicz A. and Wagner A., 1987. Micro-flora of the yellow lupine sowing material reproduced in various regions of Poland. Plant Protection, 3-5.

ISTA, **1999.** International Rules for Seed Testing. Seed Sci. Techn., 24, 5-7.

Koper R. and Dygała Z., 1994. Apparatus for the pre-sowing seed treatment with laser irradiation (in Polish). Patent RP, No. 162598.

Litwinow M.A., 1967. Microscopic Fungi Guide (in Russian). Nauka, Leningrad.

Martin I.P., 1950. Use of acid rose Bengal and streptomycin in the plate method of estimating seil fungi. Soil. Sci., 19, 215-222.

Narkiewicz-Jodko M., 1986. Phyto-pathological aspect of the cereal kernels sowing value during storage (in Polish). Zesz. Nauk AR, Wrocław, 55, 56-68.

Podleśny J., 1997. Influence of the pre-sowing seed irradiation with laser light on the formation of morphological features and faba bean yields (in Polish). Zesz. Probl. Post. Nauk Roln., 446, 435-440.

Podleśny J., 2002. Studies on the influence of laser light on seeds, growth, development and yields of white lupine (*Lupinus albus* L.) (in Polish). Monografie i Rozprawy Naukowe, IUNG-Puławy, 3.

Szyrmer J. and Klimont K., 1999. Influence of laser light on the quality of bean seeds (*Phaseolus vulgaris* L). Bulletin IGAP, 210, 165-168.