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Germination capacity and health status of hybrid alfalfa seeds after laser treatment

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A b s t r a c t. Laboratory examinations of the germination of the Polish hybrid alfalfa var. Radius were conducted completely randomly with four replications in 2002. The following factors were taken into consideration: 1) irradiation with divergent He-Ne laser beam with a surface power density in the irradiation plane of 0, 3 and 6 mW cm^{-2} applied 1, 3 and 5 times; 2) seed dressings: Funaben T, Sarfun T 65 DS and Super-Homai 70 DS in a controlled environment. The number of seeds germinating normally, those germinating abnormally, hard seeds and those infected with fungal disease were determined in the experiment.

It was found that the laser treatment of seed significantly increased the percentage of seeds germinating normally and decreased the share of seeds germinating abnormally. This had no impact on the number of hard seeds, and significantly decreased the percentage of seeds infected by fungi except for the R 6x1 dose. Hybrid alfalfa seeds were most abundantly infected by fungi of the *Penicillium* group (*Penicillium verrucosum ver. cyclopium* and *Penicillium expansum*). Seed dressings completely eliminated fungal disease in the seeds.

K e y w o r d s: laser treatment, hybrid alfalfa, germination

INTRODUCTION

Germination of hybrid alfalfa in the wild ranges from 35 to 65%, which is considerably lower than the ability of the seed to germinate as determined in laboratory tests (Ćwintal, 2000; Skrzyniarz, 1987). Germination in the wild is reduced by hard seeds and those which are diseased. In years with high temperatures and insulation and with low precipitation levels there are more hard seeds (Duczmal and Tucholska, 2000; Skrzyniarz, 1987; Vuckovic *et al.*, 1997). Soils rich in calcium, such as rendzinas, also favour a higher share of hard seeds (Wilczek, 1983). Fungi infecting seeds are divided into two groups: field and repository fungi. The field fungi include *Alternaria* and *Fusarium* and the repository group includes, for example, *Penicillium* (Duczmal and Tucholska, 2000; Narkiewicz-Jodko, 1986). The above mentioned fungi types limit the ability of the seed to germinate (Filipowicz and Wagner, 1987; Narkiewicz-Jodko, 1986). Various methods are used to improve seeds by improving basic seed parameters. One of these is the laser treatment of seed prior to sowing.

The aim of this study was to determine the influence of laser treatment of the hybrid alfalfa seed *Medicago sativa* L. ssp. *falcata x* ssp. *sativa*) on the ability to germinate, the share of hard seeds and of seeds infected with diseases in a controlled environment using seed dressings. This type of study is fully justified since no results have been quoted in the literature on the subject to date.

METHODS

Laboratory studies with hybrid alfalfa seeds were carried out in 2002. The following factors were examined: 1) irradiation with divergent He-Ne laser beam with a surface power density in the irradiation plane of 0, 3 and 6 mW cm^{-2}; 2) seed dressings: Funaben T, Sarfun T 65 DS and Super-Homai 70 DS in a controlled environment. The seeds were subjected to 1, 3 and 5 irradiation rounds on a laser device designed according to the Koper and Dygdała patent (1994). A single irradiation round lasted for 0.1 s.

Seeds of the Polish variety Radius from the 2001 harvest were used for the present study.

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Seed germination was carried out in accordance with the ISTA (1999) instructions, on Petri dishes in which 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 germinating was counted after 4 and 10 days. The number of seeds germinating normally and abnormally, as well as the number of hard seeds and seeds infected with disease was determined. The experiment was carried out completely randomly in four replications. In order to identify those fungal strains infecting the alfalfa, any seeds with mould were transferred onto a dish with Martin's (1950) substrate 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 according to the studies by Domsch (1980) and Litwinow (1967).

The results obtained were then calculated statistically using variance analysis and $LSD_{0.05}$ in accordance with Tukey's test.

RESULTS AND DISCUSSION

Hybrid alfalfa, of the Radius variety, is characterized by its relatively low ability to germinate (about 40% of the seeds germinating normally and about 17% of the seeds germinating abnormally – Table 1). Irradiation with laser beam significantly influenced the percentage of seeds germinating normally, irrespective of the surface power density dosage of the divergent laser beam and the increase in its application, except for the R 6x1 dose. The share of seeds germinating abnormally decreased significantly under the influence of laser light. The R 3x1 dose was the only exception. It should be emphasized that the variants which had been irradiated more highly exhibited a decreased share of abnormally germinating seeds than was the case where the seed had been irradiated with half the dose.

Funaben T and Super-Homai 70 DS dressings significantly increased the percentage of seeds germinating normally when compared to the control. All seed dressings tested in this experiment significantly increased the share of abnormally germinating seeds when compared to the objects in which laser treatment was applied or to the control. The total of such seeds was the highest in those objects with seed dressings (66-67%) and significantly exceeded the results obtained in the combinations with laser irradiation (52.5-61%) and the control (57%).

In the seeds of the Radius variety, a very high share of hard seeds (33-37%, Table 2) was noted. Irradiation with laser beam of various power levels and multiplications had no significant influence on the share of hard seeds after one-year of storage. The results obtained differ considerably from the respective results for seeds stored for many years (Wilczek *et al.*, 2001).

Even though seed dressings decreased the percentage of hard seeds in comparison with the control, the difference was not statistically significant. All the objects with laser treatment of the seed, except for R 6x1, were characterized by a significantly lower infection rate than was the case for the control variant.

T a b l e 1. Influence of laser treatment and seed dressing on hybrid alfalfa seed germination

Factors	% of seeds		Total of seeds germinating (%)
	germinating normally	germinating abnormally	
Irradiation dose and multiplication:			
R 0	38.0	19.0	57.0
R 3x1	42.0	19.0	61.0
R 3x3	43.8	15.0	58.8
R 3x5	42.0	17.0	59.0
Mean	42.6	17.0	59.6
R 6x1	38.5	14.0	52.5
R 6x3	44.3	16.0	60.3
R 6x5	43.3	14.0	57.3
Mean	42.0	14.7	56.7
Seed dressings:			
Funaben T	41.0	24.0	66.0
Sarfun T 65 DS	37.0	29.0	66.0
Super-Homai 70 DS	42.0	25.0	67.0
Mean	40.0	26.0	66.0
$LSD_{0.05}$	3.92	1.75	5.81

T a b l e 2. Influence of laser treatment and dressing of hybrid alfalfa on the share of hard seeds and seeds infected with disease

Factors	% of seeds		Total of seeds germinating (%)
	hard	infected with fungi	
Dose and multiplication of irradiation:			
R 0	36.5	6.5	43.0
R 3x1	34.3	4.7	39.0
R 3x3	37.0	4.2	41.2
R 3x5	36.3	4.7	41.0
Mean	35.9	4.5	40.4
R 6x1	36.0	11.5	47.5
R 6x3	36.0	3.7	39.7
R 6x5	37.0	5.7	42.7
Mean	36.3	7.0	43.3
Seed dressings:			
Funaben T	35.0	0	35.0
Sarfun T 65 DS	34.0	0	34.0
Super-Homai 70 DS	33.0	0	33.0
Mean	34.0	0	34.0
$LSD_{0.05}$	–	0.52	4.03

It is worth stressing here that seed dressings completely destroyed the fungi infecting the seed covers and significantly reduced the total of non-germinating seeds in those objects with seed dressings than was the case in the combinations with laser treatment and the control. Similar effects were also reported for the seeds of leguminous plants (Filipowicz and Wagner, 1987; Narkiewicz-Jodko, 1990).

The most frequent strains (Table 3) occurring in the seed covers of hybrid alfalfa included potentially phytopathogenic fungi of the *Alternaria* type and saprophytic fungi of

T a b l e 3. Influence of laser treatment and seed dressing on the number of fungi isolated from hybrid alfalfa seeds

Factors	Strains most frequently occurring		Number of strains isolated
	Alternaria	*Penicillium*	
Dose and multiplication of irradiation:			
R 0	2.0	14.0	20.0
R 3x1	2.0	18.0	22.0
R 3x3	2.0	19.0	23.0
R 3x5	4.0	15.0	20.0
Mean	2.7	17.3	21.7
R 6x1	4.0	31.0	39.0
R 6x3	0.0	15.0	17.0
R 6x5	2.0	20.0	23.0
Mean	2.0	22.0	26.3
Seed dressings:			
Funaben T	0	0	0
Sarfun T 65 DS	0	0	0
Super-Homai 70 DS	1.0	0	0.8
Mean	0.3	0	2.7
$LSD_{0.05}$	0.17	1.33	1.70

T a b l e 4. Influence of the increase of the irradiation dose on the germination of seed of hybrid alfalfa and its health status

	Specification	Multiplication of irradiation			Mean
		1	3	5	
1.	Percentage of seeds:				
	germinating normally	40.2	44.0	42.6	42.3
	germinating abnormally	16.5	15.5	15.5	15.8
	hard	35.1	36.5	36.6	36.1
	infected with disease	8.1	3.9	5.2	5.7
2.	Most frequently strains occurring:				
	Alternaria	3.0	1.0	3.0	2.3
	Penicillium	24.5	17.0	17.5	19.7
3.	Total number of strains isolated	30.5	20.0	21.5	24.0

the *Penicillium* type. At the same time, there were many more of this latter type of fungi represented mainly by *Penicillium verrucosum var. cyclopium* and *Penicillium expansum.* Irradiation with laser beam significantly influenced the development of fungi of the *Penicillium* type. Isolated fungi of this type were found more usually at the following doses of surface power density of the divergent laser beam in the seed irradiation plane: R 6x1; R 6x5 and R 3x3. In the case of the *Alternaria*, laser irradiation caused a significant increase in the amount of fungi but only at the doses of R 3x5 and R 6x1.

All the seed dressings completely eliminated the fungi of the *Penicillium* type, whereas no such effect was noted with respect to the *Alternaria* type, with the exception of the Super-Homai 70 DS dressing. In those objects with seed laser treatment, the highest number of fungal strains was isolated in the combination R 6x1 with R 6x5 and R 3x3. Most probably, where infestation with the *Penicillium* type of fungi is intense, seeds should be treated with seed dressings after treatment with a laser.

The percentage of seeds germinating normally was the highest with the 3 fold irradiation irrespective of the divergent laser beam power density applied (Table 4). The percentage of abnormally germinating seeds and hard seeds was similar for all the multiplications of irradiation dosage. The highest percentage of seeds infected with disease was noted at the single and the 5 fold irradiations. Undoubtedly, the highest number of fungi of the *Penicillium* type occurred with the single laser irradiation and the single and 5 fold irradiations in the case of the *Alternaria* type. The total number of strains isolated was decidedly the highest with the single irradiation. The above relations clearly show that the single irradiation process should be avoided in practice when applied to hybrid alfalfa seed, as it causes a more intense development of fungi, especially of the *Penicillium* type, which reduces the seed ability to germinate in the field (Filipowicz and Wagner, 1987; Narkiewicz-Jodko, 1986).

CONCLUSIONS

1. The treatment of seed with He-Ne laser light significantly increased the percentage of seeds germinating normally while decreasing the share of seeds germinating abnormally. The percentage of abnormally germinating seeds increased significantly under the influence of seed dressings.

2. Laser rays and seed dressings did not exert any significant influence on the share of hard seeds.

3. Laser treatment significantly decreased the percentage of those seeds infected with fungi except for the R 6x1 dose, whereas seed dressing completely eliminated fungal disease on the seeds. Fungi of the *Penicillium* genus (*Penicillum verrucosum ver. cycloplium* and *Penicillium expansum*) infected the seeds to a wider extent.

4. The laser treatment of seed should not be applied where there is widespread infection with *Penicillium* fungi.

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