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LASER LIGHT AS A PHYSICAL FACTOR ENHANCING RAPESEED RESISTANCE TO BLACKLEG DISEASE

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A b s t r a c t. The history of hardening crop plants by laser light is relatively short. After previous experimental confirmation of positive influence of laser light on seed condition, an attempt was made to use it to enhance winter rapeseed resistance to the most dangerous fungal pathogen – *Phoma lingam* (Tode ex Fr.) Desm. Such characteristics of laser beam as power, wavelength, and exposure time are of great importance for the stimulating effect. Inappropriate parameters can even cause irreparable mutation changes on the DNA level. At present, controlled seed irradiation with laser light finds practical application in seed stimulation more and more often. In this study, three different forms of winter rapeseed were used: two open pollinated varieties – Bolko and Idol, and a yellow-seeded strain (IHAR-2061/03). The seeds were irradiated with helium-neon laser at wave-length of 632 nm. The intensity of light falling on the objects was 1 mW cm^{-2}. Four exposure times were used: 30, 60, 90, and 120 min. Non-irradiated seeds were used as the control. Mycelium test was applied to check which combination had the most stimulating effect. After plant inoculation, the best results for the yellow-seeded form and variety Idol were obtained for exposure times of 30 and 60 min, and for variety Bolko – 30, 60 and 90 min. The results of this work can be used in breeding as well as in seed production to enhance rapeseed rape mechanisms of resistance to one of the principal diseases affecting this crop.

K e y w o r d s: disease resistance stimulation, laser, *Phoma lingam*, winter rapeseed

INTRODUCTION

In comparison with conventional light sources, laser is distinguished by some parameters which determine its usefulness in biological science. According to laser power and exposure time, the obtained results could have stimulating [2,7, 10,13,15] or destructive character [3,12], including changes on the DNA level

[11]. The first effect is more often successfully used in commercial scale for seed stimulation [8].

Rapeseed is the most important oilseed plant in Poland. For this reason it is very important to take special care of height and fidelity of yielding. *Phoma lingam* (Tode ex Fr.) Desm is one of the most important factors which causes decrease of yield every year [14].

Stem cancer was recognized in the last century in France [1,6]. This disease is present all over Europe, in North America, in Africa and Australia. Its first occurrence was recorded in Poland in 1952 [5]. Its appearance is observed on many species of cultivated plants among the *Cruciferae* family: broccoli, white cabbage, red cabbage, Brussels sprouts, cauliflower, kohlrabi, Italian cabbage, rapeseed, black mustard, white mustard, radish, and sarson. This disease attacks also weeds belonging to the *Cruciferae* family. The source of initial infection can be sowing material as well as spores (ascospores, conidia) arising from infected dead plant residues remaining many years after harvest [16]. Experimental data shows that in field conditions ascospores lost their vital functions only after 7 years [9]. Pycniospores (conidia) come into being on infected plants during the vegetation period. This contributes to further disease spreading. Spores germinate on the plants surface and mycelium penetrates into the plant tissues, most often by different kinds of wounds.

Investigation on the stimulation of rapeseed resistance to fungal diseases can be essential to improve yielding ability of oilseed rape.

MATERIAL AND METHODS

In this study three forms of oilseed rape were used: a yellow-seeded strain (2061/03) and two open pollinated varieties: Bolko and Idol. Seeds of *B. napus* were irradiated at the wavelength of 632 nm with helium-neon laser (especially prepared for irradiation of biological materials by Center of Laser Technique in Warsaw). The light power of the laser was 24 mW and the intensity of light falling on the objects was about 1 mW cm^{-2}. Four times of exposure were taken: 30, 60, 90, and 120 min. Non-irradiated seeds were used as the control. To check which combination would give the most stimulating effect, the mycelium test was used. This test was worked out in Poznań Department of Plant Breeding and Acclimatization Institute [14]. The method was previously successfully used for testing winter rapeseed materials for *P. lingam* disease resistance, as well as for determination of virulence of different strains of the pathogen. The test is based on rating of infection severity observed on roots and hypocotyls of rapeseed seedlings inoculated with the pathogen mycelium. Each combination was tested in two replications.

In the first step of the test, both mycelium and seedlings were grown simultaneously on different media for 7 days. Then the inoculum was evenly spread on

Petri dishes with PDA medium. (temperature 18°C). A special stamp was used to assure precise pattern of 51 infection points. During the same time, surface sterilized seeds were germinated on modified Gamborg B_5 medium [4]. Modified B_5 medium consisted of macro- and microelements and higher amount of agar (15 g/l). In solid agar, 51 holes were drilled. The diameter of the holes was similar to the diameter of seeds. The tested seeds were placed in the holes. Germination of all seeds was equal because of good air and water conditions inside the holes. In the second step, two layers of medium were merged after the 7-day preliminary growth period in such a way that each seedling was placed over one infection point. Check combinations were prepared in the same way, but without mycelium on PDA medium.

Observations of the degree of hypocotyls infection using 3-level scale were made after the next 10-14 days (scale description: 0 – lack of infection, 1 – medium infection, 2 – strong infection). The results were processed using Student's t-test.

RESULTS

The work showed that the most promising – with respect to higher rapeseed resistance to *P. lingam* inoculation – were combinations with 30-90 min (Tab. 1) exposure times for all the tested forms: yellow-seeded strain, and varieties Idol and Bolko (Photo. 1-4).

Table 1. Number of plants after irradiation which were resistant to *P. lingam*

Exposure time (min)	Variety					
	2061/03 yellow-seeded form		Bolko		Idol	
	Number of resistant plants	Number of non-resistant plants	Number of resistant plants	Number of non-resistant plants	Number of resistant plants	Number of non-resistant plants
Control	10	92	18	84	15	87
30	27	75	43	59	30	72
60	26	76	38	64	29	73
90	24	78	48	54	18	84
120	10	92	38	64	18	84
Total plants	97	413	185	325	110	400

All comparisons between the tested combinations and the control were statistically significant. The best protection results were obtained at 30 min exposure time (Student's t-test $P < 8.1 \cdot 10^{-5}$ **). Similar effects were obtained for 60 min exposure time (Student's t-test $P < 7.7 \cdot 10^{-4}$ **) and 90 min exposure time

(Student's t-test $P < 7{,}7 \cdot 10^{-3}$ **). 120 min exposure time gave statistically less significant results (Student's t-test $P < 3.7 \cdot 10^{-2}$ *).

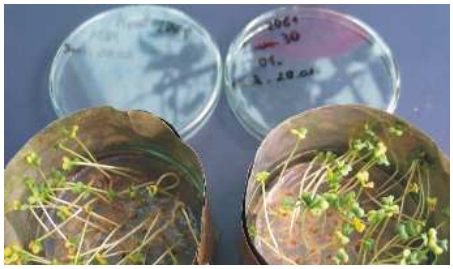

Photo 1. Yellow-seeded rapeseed plants 14 days after *P. lingam* inoculation
On the left – control plants derived from non-irradiated seeds
On the right – plants derived from seeds after 30 min laser irradiation

Photo 2. Idol rapeseed plants in 14 days after *P. lingam* inoculation
On the left – control plants derived from non-irradiated seeds
On the right – plants derived from seeds after 30 min laser irradiation

Photo 3. Bolko rapeseed plants 14 days after *P. lingam* inoculation.
On the left – control plants derived from non-irradiated seeds
On the right – plants derived from seeds after 60 min laser irradiation

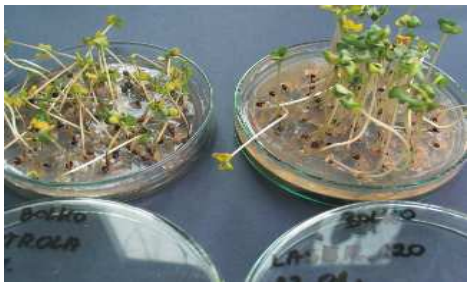

Photo 4. Bolko rapeseed plants 14 days after *P. lingam* inoculation.
On the left – control plants derived from non-irradiated seeds
On the right – plants derived from seeds after 120 min laser irradiation

DISCUSSION

These preliminary results show that laser light irradiation can be useful for improving rapeseed yield performance in the field conditions, especially in those regions where the blackleg disease occurs.

The investigation should be extended onto different natural environments and other rapeseed pathogens. It should be pointed out that laser light is widely applied in horticulture not only for improving plant resistance, but also for

enhancing green mass and seed productivity [8]. The mycelium test for the evaluation of rapeseed resistance, which was used in this investigation, is a very sensitive and repeatable method.

The best results of positive influence of laser light on seedlings health were obtained in the combinations of 30-90 min exposure time (Photo. 1-3). 120 min irradiation was less efficient with respect to the tested trait (Photo. 4). Too long irradiation can cause considerable damage to the seed structures. There was no significant difference between variety reactions to laser exposure. The most resistant variety turned out to be Bolko. The least resistant was the yellow-seeded strain. These differences depend on the genetic character of the varieties.

CONCLUSION

Stimulating effect of helium-neon laser light can be used in breeding and high quality seed production of winter rapeseed. The best results were obtained at 30-90 min irradiation time at exposure of 1 $mW \cdot cm^{-2}$. So these conditions are recommended for practice.

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ŚWIATŁO LASERA JAKO FIZYCZNY CZYNNIK WSPOMAGAJĄCY ODPORNOŚĆ RZEPAKU OZIMEGO NA SUCHĄ ZGNILIZNĘ ROŚLIN KAPUSTNYCH

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S t r e s z c z e n i e. Historia kondycjonowania laserem roślin rolniczych jest stosunkowo krótka. Po eksperymentalnym stwierdzeniu dobroczynnego wpływu światła lasera na nasiona podjęto próby wykorzystania go do indukowania odporności rzepaku ozimego na najgroźniejszego patogena grzybowego, jakim jest *Phoma lingam* (Tode ex Fr.) Desm. Moc, długość fali i czas ekspozycji lasera mają decydujące znaczenie dla otrzymania efektów biostymulujących. Nieodpowiednio dobrane parametry mogą powodować nieodwracalne zmiany mutacyjne na poziomie DNA. Naświetlanie nasion światłem lasera w sposób kontrolowany coraz częściej znajduje praktyczne zastosowanie do biostymulacji nasion. W badaniach wykorzystano trzy formy rzepaku ozimego: dwie odmiany populacyjne Bolko i Idol oraz ród żółtonasienny (IHAR-2061/03). Nasiona naświetlano laserem helowo-neonowy, o długości fali 632 nm. Moc światła padającego na naświetlane obiekty wynosiła 1 $mW \cdot cm^{-2}$. W badaniach efektu biostymulacji wykorzystano cztery czasy ekspozycji: 30, 60, 90 i 120 minut. Nasiona bez doświetlania laserem stanowiły kontrolę. Aby stwierdzić, która z kombinacji jest najlepsza dla stymulacji odporności rzepaku na *P. lingam*, wykorzystano metodę grzybniową. Po inokulacji roślin, najlepszą wersją, na której zaobserwowano podwyższoną odporność u formy żółtonasiennej i odmiany Idol, była kombinacja z czasem ekspozycji lasera 30 i 60 minut, a dla odmiany Bolko 30, 60 i 90 minut. Przedstawiona praca może zostać wykorzystana aplikacyjnie w hodowli i nasiennictwie rzepaku ozimego w celu podwyższenia jego odporności na najgroźniejszą chorobę, jaką jest sucha zgnilizna roślin kapustnych.

S ł o w a k l u c z o w e: stymulacja odporności, laser, *Phoma lingam*, rzepak ozimy