

**RELATIONSHIP BETWEEN THE LEVEL
OF CARBOHYDRATES AND SUSCEPTIBILITY OF YELLOW
LUPIN EMBRYO AXES TO
Fusarium oxysporum SCHLECHT. f. sp. *lupini* INFECTION**

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* The author has received a stipend from the Foundation for Polish Science

Introduction

At the early stages of seed germination, an unfavourable effect of environmental factors and deep sowing are conducive to the prevalence of fungal infections. The most common fungal diseases of lupin sprouts and seedlings are fusarioses, the first symptoms of which are visible at the time of plant emergence, which raises a question whether the level of carbohydrates in seeds may play an important role in plant defense against fungal diseases. At the initial stage of germinating seeds, the consumption of carbohydrates for respiratory processes is intensive. A significant loss of carbohydrates in the germinating seed axis leads to the so-called carbohydrate starvation, which lasts up to the moment before the developing seedling starts independent sugar synthesis [YU 1999; MORKUNAS et al. 2000]. Particularly susceptible to this kind of starvation are contemporary yellow lupin cultivars, the selection of which conducted with the aim to increase protein has caused a simultaneous drop in soluble sugars in seeds. Carbohydrates, especially oligosaccharides, are ascribed to have a significant role in the induction of plant resistance to pathogenic fungi [SHARON 1995]. There are many pieces of evidence that carbohydrates may play an important role in plant defense against diseases because they enhance synthesis of phenols, phytoalexins and lignins [KUC 1995; RAVN et al. 1998]. Particularly interesting in this respect are the cognition of the role of carbohydrates in plant-pathogen interactions and tracing of the consequent metabolic and biochemical changes as an effect of their actions. Model studies as well as observations conducted on plants have showed the existence of rapid plant defense reactions to pathogen infections. They include an oxidative burst, in which the dominant role is played by hydrogen peroxide and superoxide radical, whereas hydroxyl radical activates a cascade of radical reactions. A high concentration of free radicals in cells may primarily cause the inhibition of mycelium germination and growth, however, if it lasts longer, that may bring about cell damage [MAŁOLEPSZA, URBANEK 2000; DAT et al. 2000]. Assuming that plants have developed an array of constitutive and induced defense mecha-

nisms which prevent or restrict pathogen invasion, the aim of this work was to determine the level of free radicals, which are the earliest responses of plant cells to infection.

Material and methods

The initial material for the studies were cv. Pole seeds of yellow lupin (*Lupinus luteus* L.) constituting a super elite. The experiments were carried out on *in vitro* cultured embryo axes isolated from swelling seeds, which after cotyledon excision were deprived of their nutrient reserves and subjected to the medium carbon source. Then, yellow lupin tissues were infected with *Fusarium oxysporum* f. sp. *lupini*. Four variants of tissue culture were applied: 1) non-infected tissues grown on Heller medium with 60 mmol·dm⁻³ sucrose, 2) infected tissues grown on Heller medium with 60 mmol·dm⁻³ sucrose, 3) non-infected tissues grown on the medium without sugar and 4) infected tissues grown on the medium without sugar. The tissues were taken for analyses after 24, 48, 72 and 96 hours of culture.

Fungi and inoculation

The *Fusarium oxysporum* f. sp. *lupini* strain K-1018 was obtained from the Bank of Pathogens, Institute of Plant Protection, Poznań. The strain was grown on PDA medium (potato-dextrose-agar, Difco) in the light at the temperature of 24°C. The inoculum (spore suspension) used for infecting the tissues was prepared from a 3-week-old culture and was applied at a concentration of 5·10⁶ spores per 1 ml.

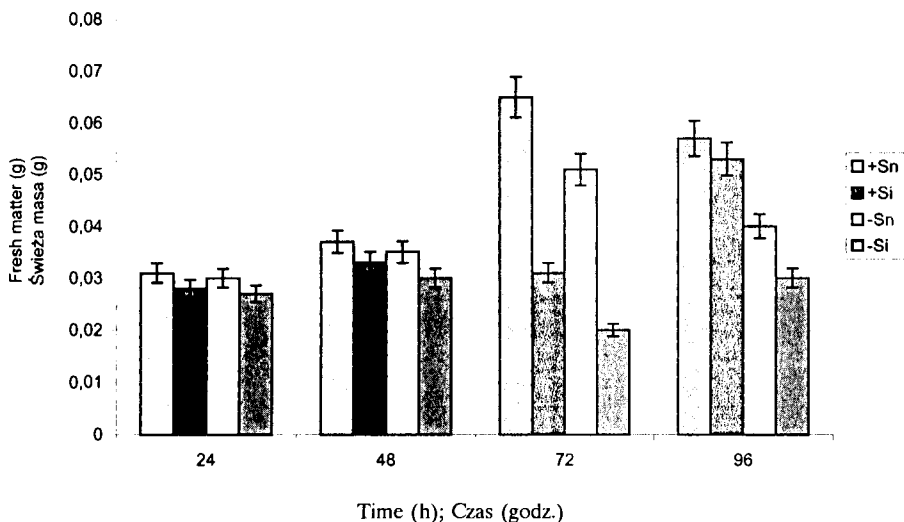
Electron paramagnetic resonance (EPR)

Samples of 24 embryo axes of the above culture variants were frozen in liquid nitrogen, lyophilized in the freeze dryer Jouan LP3, and ground in mortar. The ground material was introduced into EPR-type quartz tubes (4 mm in diameter). The concentration of free radicals and Mn²⁺ ions was measured in the embryo axes for 24, 48, 72 and 96 hours applying EPR spectrometer, which recorded signals at X-band (microwave frequency – 9.4 GHz). The EPR signal was monitored by measuring the first derivative of microwave absorption. The line amplitude of each sample and line width of EPR lines after computer numerical double integration of the first derivative curve [AYSCOUGH 1967] enabled the total spin intensity of each sample to be recorded under identical conditions. Since the samples were of equal volume, but of different weight, the areas under the EPR curve per 1 mg of each sample were calculated.

Results and Discussion

Treatment of lupin embryo axes with *Fusarium oxysporum* f. sp. *lupini* spores suspension has caused a decrease in the material fresh weight (Fig. 1). That

decrease was especially visible in the case of a 72-hour culture, where it amounted to about 60% in comparison to the non-infected material. This result was the effect of progressing fusarium rot and necrosis, which are common on legume seedlings. *Fusarium* fungi cause both pre- and post-emergence necrosis and infect seeds damaging them or decreasing their ability to germinate and inducing mitotoxin infection [BORECKI 1987].



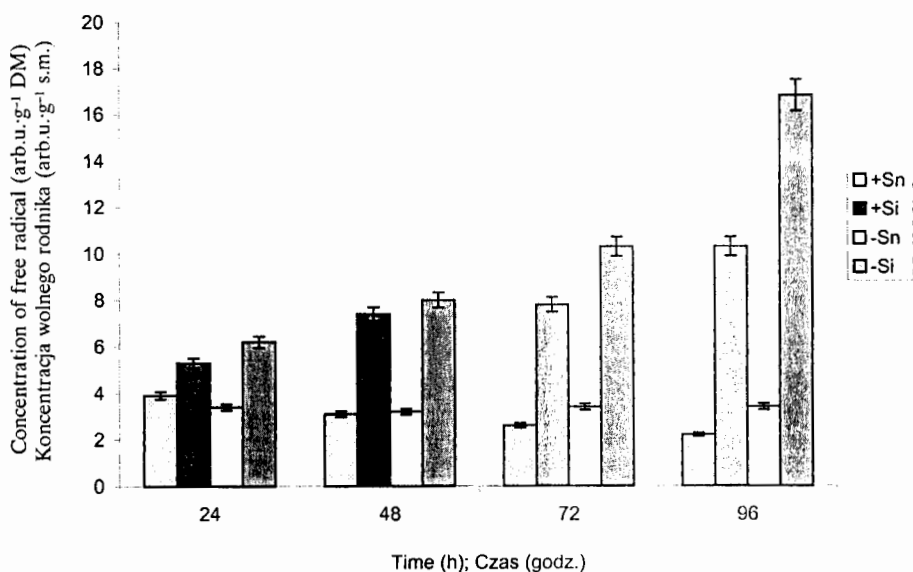
- +Sn – non-infected embryo axes cultured on Heller medium with 60 mmol·dm⁻³ sucrose; osie zarodkowe nieinfekowane hodowane na pożywce Hellera z 60 mmol·dm⁻³ sacharozą
- +Si – infected embryo axes cultured on Heller medium with 60 mmol·dm⁻³ sucrose; osie zarodkowe infekowane hodowane na pożywce Hellera z 60 mmol·dm⁻³ sacharozą
- Sn – non-infected embryo axes cultured on medium without sugar; osie zarodkowe nieinfekowane hodowane na pożywce bez cukru
- Si – infected embryo axes cultured on medium without sugar; osie zarodkowe infekowane hodowane na pożywce bez cukru

Fig. 1. Differences in fresh weight of yellow lupin embryo axes infected with *Fusarium oxysporum* f. sp. *lupini* spores, cultivated *in vitro* on medium with and without sucrose. The data are given as means of four independent experiments. Twenty-four embryo axes from each culture variant were taken for measurement. Fresh weights are given in grams per one embryo axis

Rys. 1. Różnice w świeżej masie osi zarodkowych łubinu infekowanych zarodnikami *Fusarium oxysporum* f. sp. *lupini* hodowanych *in vitro* na pożywce z sacharozą i bez cukru. Przedstawione dane są średnią z czterech niezależnych eksperymentów. Do oznaczeń brano po 24 osie zarodkowe z każdego wariantu. Świeżą masę podawano w gramach w przeliczeniu na 1 oś zarodkową

In view of the fact that stress factors may increase synthesis of reactive oxygen forms, we determined the concentration of free radicals by the method of electron paramagnetic resonance. Measurements of free radical concentration presented in Fig. 2 showed the presence of free radicals with the coefficient g

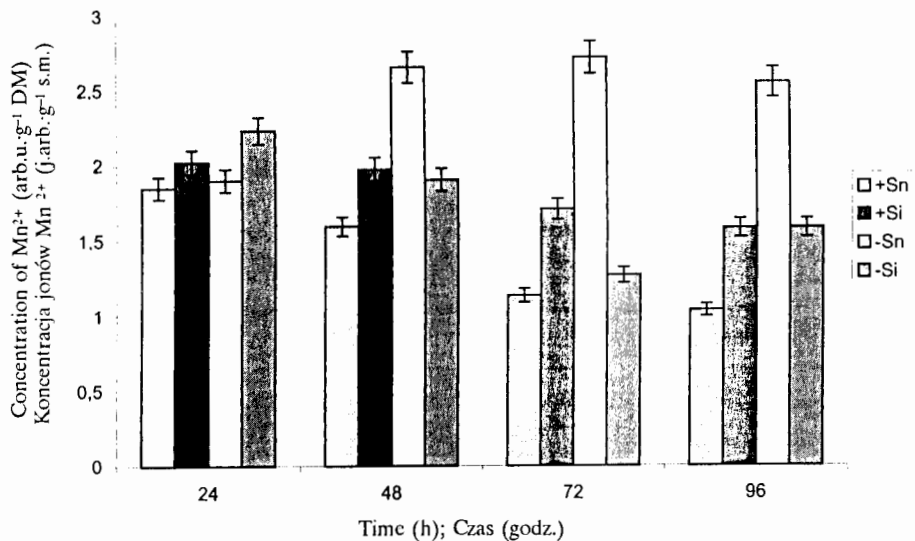
value of 2.0037 in lupin embryo axes. The level of that free radical was significantly higher in the material infected with *Fusarium oxysporum* f. sp. *lupini* spores than in the non-infected material. Differences in the free radical concentration between the infected and non-infected materials intensify with the passage of the culture time. The highest, 4-fold increase of the free radical concentration was observed under the influence of infection in the embryo axes cultured for 96 hours both on the medium with and without sucrose. Simultaneously with measurements of the free radical concentration, the concentration of Mn^{+2} ions was also measured. When analysing the concentration of Mn^{+2} in lupin embryo axes cultured on the medium with sucrose, their concentration was found to increase under the influence of infection, which particularly concerned the infected tissues 72 and 96 hours after inoculation (Fig. 3).



- +Sn – non-infected embryo axes cultured on Heller medium with 60 mmol·dm⁻³ sucrose; osie zarodkowe nieinfekowane hodowane na pożywce Hellera z 60 mmol·dm⁻³ sacharozy
- +Si – infected embryo axes cultured on Heller medium with 60 mmol·dm⁻³ sucrose; osie zarodkowe infekowane hodowane na pożywce Hellera z 60 mmol·dm⁻³ sacharozy
- Sn – non-infected embryo axes cultured on medium without sugar; osie zarodkowe nieinfekowane hodowane na pożywce bez cukru
- Si – infected embryo axes cultured on medium without sugar; osie zarodkowe infekowane hodowane na pożywce bez cukru

Fig. 2. Concentration of the free radical in yellow lupin embryo axes infected and non-infected with *Fusarium oxysporum* f. sp. *lupini* cultivated for 24, 48, 72, and 96 hours on medium with and without sugar. The data are given as means of four independent experiments.

Rys. 2. Koncentracja wolnych rodników w osiach zarodkowych łubinu infekowanych i nieinfekowanych *Fusarium oxysporum* f. sp. *lupini* hodowanych przez 24, 48, 72, 96 godzin na pożywce z sacharozą i bez cukru. Przedstawione dane są średnią z czterech niezależnych eksperymentów



- +Sn – non-infected embryo axes cultured on Heller medium with 60 mmol·dm⁻³ sucrose; osie zarodkowe nieinfekowane hodowane na pożywce Hellera z 60 mmol·dm⁻³ sacharozy
- +Si – infected embryo axes cultured on Heller medium with 60 mmol·dm⁻³ sucrose; osie zarodkowe infekowane hodowane na pożywce Hellera z 60 mmol·dm⁻³ sacharozy
- Sn – non-infected embryo axes cultured on medium without sugar; osie zarodkowe nieinfekowane hodowane na pożywce bez cukru
- Si – infected embryo axes cultured on medium without sugar; osie zarodkowe infekowane hodowane na pożywce bez cukru

Fig. 3. Concentration of manganese ions in infected and non-infected embryo axes of lupin cultured *in vitro* for 24–96 hours on the medium containing either 60 mmol sucrose or no sugar. The data are given as means of four independent experiments.

Rys. 3. Koncentracja jonów manganu w infekowanych i nieinfekowanych osiach zarodkowych łubinu hodowanego *in vitro* przez 24–96 godzin na pożywce z sacharozą i bez cukru. Przedstawione dane są średnią z czterech niezależnych eksperymentów

An intensified generation of free radicals in the infected material is one of essential symptoms of the plant cell defense response to a pathogen attack [DEIGHTON et al. 1999]. This process is associated with the so-called oxidative burst, which belongs to the first stage of plant response activated at the moment of pathogenic micro-organism attack. Responses of that type were found also in the case of plant – pathogen interaction in *in vitro* model and *in vivo* cultures [BAKER, ORLANDI 1995; KOZŁOWSKA, FLORYSZAK-WIECZOREK 1995; MEHDY 1994; BOLWELL et al. 1998]. When comparing also the free radical concentration in the material infected with *Fusarium oxysporum* f. sp. *lupini* cultured under different trophic conditions, it was found that the free radical level in lupin embryo axes increased in the absence of carbohydrates. This inclines to the suggestion that the oxidative burst causes cell damage, which may be favourable to the

development of fungal infection. It is known from the literature that well sugar-fed tissues are more resistant to a pathogen attack than tissues with sugar deficiency [VIDHYASEKARAN 1988]. The role of sugars in the resistance to a pathogen attack is supported by results obtained by SKRABKA and PRZYBYLSKA [1983], which indicate that resistance to fusariosis depends rather on the type of simple sugar accumulation than on its amount. In this connection we have suggested that the resistance mechanism of sugar-fed tissues consists in levelling toxic effect of free radicals, since the presence of carbohydrates decreased their concentration. We suggest that carbohydrates are conducive to the synthesis of numerous phenolic compounds, which can reduce reactive oxygen forms, fulfilling the role of free radical scavengers. As a result of the above oxidative reactions, phenolic compounds convert into metabolites with antimicrobial properties. A precise understanding of that mechanism requires further complex investigations.

Conclusions

1. Tissue infection with *Fusarium oxysporum* f. sp. has caused a decrease in the fresh weight of yellow lupin embryo axes.
2. Generation of free radical with the g value of 2.0037 has been found to increase as a result of infection in the cells of yellow lupin embryo axes.
3. We have suggested that the resistance mechanism of lupin sugar-fed tissues consists in levelling free radical toxic effect.

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Key words: carbohydrates, electron paramagnetic resonance, embryo axes, *Fusarium oxysporum* SCHLECHT. f. sp. *lupini*, free radicals, infection, *Lupinus luteus* L.

Summary

The aim of the studies was to examine whether carbohydrates being an important nutritious agent and releasing a metabolic signal may play an essential role in plant resistance to fungal infections. At the early stages of seed germination in legume plants there occurs an intensive carbohydrate consumption for respiratory processes. A significant loss of carbohydrates in the germinating seed axis leads to the so-called carbohydrate starvation, which lasts up to the moment before the developing seedling starts independent sugar synthesis. Contemporary breeding cultivars of yellow lupin are especially subjected to carbohydrate starvation, because their selection conducted with the aim to increase protein content, has consequently caused a simultaneous drop in soluble sugars in seeds. In the earliest period of development, unfavourable environmental conditions as well as deep sowing are conducive to the occurrence of fungal infections. The most common fungal diseases of seedlings are fusarioses, the first symptoms of which are visible already at the time of plant emergence. So, it seems that the level of carbohydrates in seeds may play an important role in plant defense against diseases. Assuming that plants have developed a wide range of constitutive and induced

defense mechanisms preventing or restricting pathogen invasion, the purpose of the present studies was to determine whether the level of carbohydrates influences plant response at the moment of pathogenic fungal attack. Since it is known that one of the first plant cell responses to pathogen invasion is the so-called oxidative burst generating free radicals, the present paper contains results of determinations of the level of free radicals and Mn^{+2} ions in yellow lupin embryo axes infected with *Fusarium oxysporum* f. sp. *lupini*. The experiments were performed on the embryo axes of yellow lupin, which after the infection were cultured *in vitro* for 24–96 hours on medium with 60 mmol·dm⁻³ sucrose and without sugar. Results of free radical measurements made by EPR method have showed the presence of free radicals the level of which was higher in the embryo axes infected with the spores of *Fusarium oxysporum* f. sp. *lupini* than in the non-infected material. Comparing also the free radical concentration in the material infected with *Fusarium oxysporum* f. sp. *lupini* cultured under different trophic conditions, it has been found that the free radical level in the lupin embryo axes increased at the lack of carbohydrates. Lupin embryo axes cultured on medium with sucrose have been found to have an increase of Mn^{+2} ions concentration under the influence of infection, particularly after 76 and 96 hours of culture. The obtained results suggest that the resistance mechanism of sugar-fed tissues consists in levelling free radical toxic effect.

ZALEŻNOŚĆ POMIĘDZY POZIOMEM WĘGLOWODANÓW A WRAŻLIWOŚCIĄ OSI ZARODKOWYCH ŁUBINU ŻÓŁTEGO NA INFEKCJE *Fusarium oxysporum* SCHLECHT. f. sp. *lupini*

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Słowa kluczowe: węglowodan, yelektronowy rezonans paramagnetyczny, osie zarodkowe, *Fusarium oxysporum* SCHLECHT. f. sp. *lupini*, infekcja, *Lupinus luteus* L., wolne rodniki

Streszczenie

Celem badań było sprawdzenie czy węglowodany będące ważnym czynnikiem odżywczym oraz wywołające sygnał metaboliczny mogą odgrywać istotną rolę w odporności roślin na infekcje grzybowe. We wczesnych fazach kiełkowania nasion roślin motylkowych następuje intensywne zużycie węglowodanów do procesów oddechowych. Znaczny ubytek węglowodanów w osi kiełkującego nasienia prowadzi do tzw. głodu węglowodanowego, który trwa aż do momentu zanim rozwijająca się siewka rozpocznie samodzielną syntezę cukrów. Na głód ten narażone są szczególnie współcześnie hodowane odmiany łubinu żółtego, których selekcja prowadzona pod kątem zwiększenia poziomu białek spowodowała równoległy spadek cukrów rozpuszczalnych w nasionach. W tym najwcześniejszym okresie rozwoju, niekorzystne warunki środowiskowe oraz głęboki siew nasion

sprzyjają rozwojowi infekcji grzybowych. Najczęściej występującymi chorobami grzybowymi siewek są fuzariozy, których pierwsze objawy są widoczne już w czasie wschodów roślin. Wydaje się zatem, że poziom węglowodanów w nasionach może odgrywać ważną rolę w obronie organizmu przed chorobami. Przyjmując, że rośliny stosują szeroki arsenał konstytutywnych i indukowanych mechanizmów obronnych zapobiegających lub ograniczających inwazję patogenów, celem niniejszej pracy było określenie czy poziom węglowodanów wpływa na reakcje roślin w chwili ataku grzybów chorobotwórczych. Ponieważ wiadomym jest, że jedną z pierwszych odpowiedzi komórek roślinnych na inwazję patogena jest tzw. wybuch tlenowy generujący wolne rodniki, w niniejszej pracy przedstawiono wyniki oznaczeń poziomu wolnych rodników i jonów Mn^{+2} w osiach zarodkowych łubinu żółtego infekowanych *Fusarium oxysporum* f. sp. *lupini*. Doświadczenia przeprowadzono na osiach zarodkowych łubinu żółtego, które po infekcji hodowano w czasie od 24 do 96 godzin in vitro na pożywce z 60 mmol·dm⁻³ sacharozy i bez cukru. Wyniki pomiaru wolnych rodników metodą EPR wykazały obecność wolnego rodnika, którego poziom w osiach zarodkowych infekowanych zarodnikami *Fusarium oxysporum* f. sp. *lupini* był wyższy w stosunku do materiału nieinfekowanego. Porównując także stężenie wolnego rodnika w materiale infekowanym *Fusarium oxysporum* f. sp. *lupini* hodowanym w różnych warunkach troficznych stwierdzono, że przy braku węglowodanów następował w osiach zarodkowych łubinu wzrost poziomu wolnego rodnika. W osiach zarodkowych łubinu hodowanych na pożywce z sacharozą stwierdzono wzrost stężenia jonów Mn^{+2} pod wpływem infekcji, szczególnie po 72 i 96 godzinach hodowli. Uzyskane wyniki sugerują, że mechanizm odporności tkanek odżywionych cukrem, polega na niwelowaniu toksycznego działania wolnych rodników.

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