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# BIOCHEMICAL MARKERS OF WHEAT EYESPOT RESISTANCE

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## Abstract

The change of lipid peroxidation (LPO) intensity during pathogenesis was used for the estimation of wheat plant resistance to the causal agent of eyespot. Seeds were infected by conidia of highly virulent strain. Sensitive and resistant varieties were investigated.

The control plants contained maximum malone dialdehyde (MDA) on the first day after germination and then MDA content decreased. The infected plants had maximum MDA content on the second day – it was higher for sensitive variety than for resistant. Morphometric indicators were higher for infected plants.

The data obtained enable us to recommend the analysis of LPO change as the most objective method of estimation of wheat resistance to the causal agent of eyespot.

Key words: lipid peroxidation, wheat plants, causal agent of eyespot, resistance.

## Introduction

Phytopathogenic micro-organisms, which attack cultivated crops, inflict considerable harm for agricultural production. Winter wheat grain losses to diseases amount to 18–20%. The danger of different fungal diseases to crops has increased considerably under the conditions of satiation of crop rotations by grain-crops, and growing of wheat under intensive technology. According to the data of the Institute of Plant Protection (Kyiv, Ukraine) eyespot, take-all, *helminthosporium* and f*usarium* are common winter wheat diseases and account for 12% of the total wheat diseases. Eyespot is the most dangerous disease.

Eyespot is a primary cause of lodging of crops. The disease results in disruption of nutrient and water translocation, leading to a weakening and finally breaking of the stem base. The quality of grain deteriorates because the fungus interrupts nutrient transport. Yield losses due to eyespot infestation can be as high as 40–50% /Жалиева, 2005/. Eyespot is widespread all over the country but inflicts most harm in Polissya, Western and Central Forest-steppe, Steppe, on irrigation.

Eyespot is caused by fungus *Oculimacula yallundae* /Wallwork, Spooner, 1988; Crous et al., 2003/. Infected stubble is the source of infection. Infection is not passed with grain /Тютерев, 2001/. There are no eyespot immune varieties /Левитин, Тютерев, 2003/. Knowledge of biochemical nature of protective reactions and peculiarities of host-plant and pathogen interaction raise the need for the development and use of eyespot resistant varieties.

The main aim of the current study was to investigate a possibility to use the analysis of lipid peroxidation (LPO) change as an indicator of wheat resistance to the causal agent of eyespot.

**Materials and Methods**

Winter wheat (*Triticum aestivum* L.) varieties of different ecological groups: poliska (ʻPoliska 29ʼ, ʻPoliska 90ʼ), forest-steppe (ʻMironivska 808ʼ), steppe (ʻAlmazʼ, ʻLada Odeskaʼ, ʻShostipalivkaʼ, ʻOdeska 269ʼ, ʻTronka, ʻKhersonskaʼ) and the variety of the French breeding (ʻRoazonʼ) were tested in the experiments. Highly virulent strain 543 7/1 of *Oculimacula yallundae* was used. The fungi were grown on a liquid potato-glucose nutrient medium amended with gentamycin sulphate (80 mg $L^{-1}$) (Figure 1).

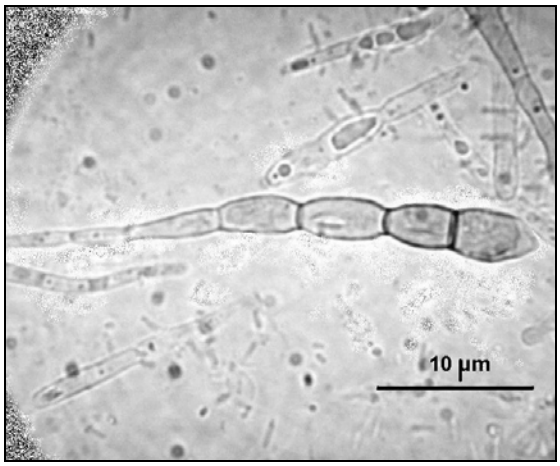


***Figure 1.*** *Oculimacula yallundae* conidium

Eyespot is a stem base disease, therefore inoculum must be added into the soil. In our experiments, wheat seeds were infected by conidia suspension and inoculum was added to the sand. The seeds were sterilized by 2% $KMnO_4$, carefully washed by distilled water, laid out on a sterile filter paper in Petri dishes, and distilled water was added. Dishes with vegetable material were transferred to a thermostat for 24 hours at $24^o$ C. After that, water surplus was poured off and the same amount of conidia suspension of *Oculimacula yallundae* (initial concentration 5–7 x $10^4$ conidia $mL^{-1}$) was added and placed in a thermostat for 8 hours. A new portion of distilled water was added in the control treatment. Containers (9 x 8 x 8 cm) were filled with quartz sand to 4/5 height. 100 ml of Khoglend-Arnon nutrient medium /Гродзинский А.М., Гродзинский Д. М., 1973/ was poured in each container. Germinating seeds were laid on moist sand (64 seeds per container). Conidia suspension of *Oculimacula yallundae* was laid on every infected seed (twice, for 2 drops). In the control, seeds were moistened with distilled water. All seeds were covered by sand (thickness of cover of 0.5 cm). Containers were placed in separate pallets. Khoglend-Arnon nutrient medium was poured in every pallet. Plant material grew at a 16-hour photoperiod, illumination $6x10^3$ luces. Nutrient medium was added for pallets according to the need.

Level of products of LPO, indexes of integral growth processes, content of photosynthetic pigments were determined for the estimation of wheat varieties' resistance to *Oculimacula yallundae*.

LPO level was estimated on content of malone dialdehyde (MDA), which was determined after reaction with 2-thiobarbituric acid and measured on a spectrophotometer SF-46 at a wave-length 533 nm /Мерзляк и др., 1979/.

The dynamics of indicators of integral growth processes (length, fresh and dry weight of above-ground part; length, fresh and dry weight of roots) was determined according to the common methods.

The content of pigments (chlorophyll *a* and *b*, carotenes) was determined on a spectrophotometer in 100% acetone /Arnon, 1949/.

Results were statistically processed /Доспехов, 1985/. The differences between the experimental treatments were significant at ≤ 5% significance level after the Student test.

**Results and Discussion**

Infecting of plants by pathogenes is a stress, which causes a cascade of metabolic changes. One of them – formation and accumulation of active oxygen forms (oxidative burst), cause oxidative damage of plant cells, which results in LPO /Шакирова, 2001/. LPO intensity change is a heterospecific response to the abiotic /Жиров и др., 1982; Лукаткин, Голованова, 1988; Платонова, Остишин, 2000/ and biotic /Жуков и др., 2001, Шакирова, 2001/ stress. That is why LPO intensity change (the accumulation of MDA) during pathogenesis was used to study wheat plant resistance to the causal agent of eyespot. Ten wheat varieties were tested. Varieties with the least increase of MDA content in relation to control (Table) – 'Mironivska 808' (sensitive), 'Roazon', 'Almaz', 'Shostipalivka' (relatively resistant) were chosen.

***Table.*** Effects of Oculimacula yallundae on MDA content in winter wheat varieties (P≤5)

| Variety | MDA content, mmol $g^{-1}$ | | |
|---|---|---|---|
| | control | infected | % to the control |
| Mironivska 808 | 0.571 | 0.949 | 166.200 |
| Roazon | 0.470 | 0.569 | 121.064 |
| Almaz | 0.503 | 0.546 | 108.548 |
| Lada Odeska | 0.507 | 0.786 | 155.030 |
| Shostipalivka | 0.420 | 0.630 | 150.000 |
| Odeska 269 | 0.521 | 0.813 | 156.046 |
| Poliska 29 | 0.527 | 0.838 | 159.013 |
| Poliska 90 | 0.531 | 0.855 | 161.017 |
| Tronka | 0.526 | 0.831 | 157.985 |
| Khersonska | 0.528 | 0.835 | 158.144 |

The control plants of all varieties contained maximum MDA on the first days after germination and then MDA content decreased.

In the sensitive variety ʻMironivska 808ʼ MDA content declined by 32% to the second day and then increased unessentially to the fifth day (Figure 2).

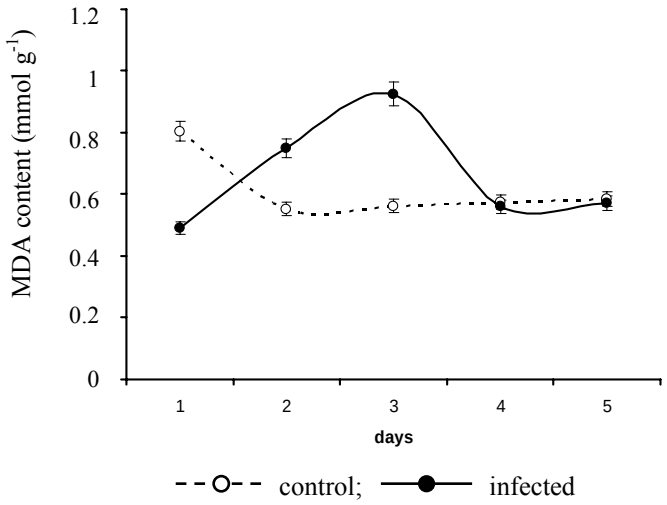


***Figure 2.*** MDA content dynamics in wheat seedlings of the sensitive variety ʻMironivska 808ʼ

In relatively resistant varieties (ʻAlmazʼ, ʻShostipalivkaʼ, ʻRoazonʼ) MDA content decreased till the fifth day by 50–56%, MDA minimum content was observed on the third day, it was 42–51% from the initial level (Figure 3).

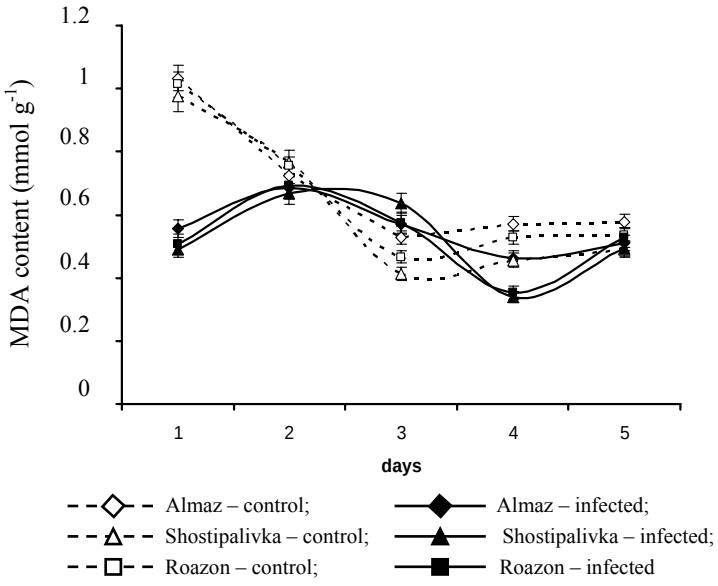


***Figure 3.*** MDA content dynamics in wheat seedlings of relatively resistant varieties

MDA content in the infected plants of all varieties was 50–61% to the control on the first day. Maximum MDA content for the infected sensitive variety marked on the third day was 165% to the control and by 1.9 times exceeded the initial level. Maximum MDA content for the infected relatively resistant varieties marked on the second day was 87–95% to the control and by 1.23–1.36 times exceeded the initial level. Minimum MDA content for the infected plants of all varieties tested was observed on the fourth day after seed germination. It was 69–83% to the initial level for the infected plants of relatively resistant varieties. On the fifth day, the MDA amount for the infected plants of relatively resistant varieties was almost the same as on the first day.

The data obtained confirms transgression of lipid exchange under pathogen pressure action. It was established that MDA level for the infected plants of all varieties on the second day was approximately 0.7 mmol $g^{-1}$. On the third day, this indicator diminished for relatively resistant varieties ʻRoazonʼ, ʻAlmazʼ, ʻShostipalivkaʼ and continued to grow in the sensitive variety ʻMironivska 808ʼ. It is likely that the increase of MDA level, as LPO product, during infection is a consequence of destruction of membrane lipids, which results in partial membrane disorganization and increase of their permeability /Гуральчук, 1994/, necessary for successful distribution of infection. Subsequent sharp reduction in MDA content (even below initial) may be the result of protective reactions start in plant cells directed to maintenance of homoeostasis under infection to prevent development of irreversible pathological changes.

Changes of chlorophyll *a* and *b* content in the infected plants of relatively resistant varieties were insignificant, while in sensitive varieties chlorophyll *a* amount was 126% to the control, and chlorophyll *b* – 270% to the control. The infection did not influence carotenoid content.

Indicators of integral growth processes for the sensitive variety ʻMironivska 808ʼ under infection were higher, than for the control. For the infected plants of relatively resistant varieties these indicators varied from 79.71% to 124.98% to the control. This is likely to be connected with the secretion of stimulating compounds by pathogen at the initial stages of infection development.

## Conclusions

The data obtained allow us to recommend the analysis of LPO change (the accumulation of MDA) as the most objective method of estimation of wheat resistance to the causal agent of eyespot at the early stages of development.

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## REFERENCES


1. Arnon D. Copper enrymen in isolated chloroplasts. Polyphenol oxidase in Beta Vulgaris // Plant Physiology. – 1949, vol. 24, p. 1–15

2. Crous P. W., Groenewald J. Z., Gams W. Eyespot of Cereals Revisited: ITS phylogeny Reveals New Species Relationships // European Journal of Plant Pathology. – 2003, vol. 109, No. 8, p. 841–850

3. Wallwork H., Spooner B. Tapesia yallundae – The teleomorph of Pseudocercosporella herpotrichoides // Transactions of the British Mycological Society. – 1988, vol. 91, No. 44, p. 703–705

4. Гродзинский А. М., Гродзинский Д. М. Краткий справочник по физиологии растений. – Киев, 1973. – 576 с.

5. Гуральчук Ж. З. Механизмы устойчивости растений к тяжелым металлам // Физиология и биохимия культурных растений. – 1994, т. 26, № 2, с. 107–117

6. Доспехов Б. А. Методика полевого опыта (с основами статистической обработки результатов исследований). – Москва, 1985. – 315 с.

7. Жалиева Л. Д. Факторы, формирующие комплекс возбудителей гнилей озимой пшеницы // Защита и карантин растений. – 2005, № 1, с. 26

8. Жиров В. К., Мерзляк М. Н., Кузнецов Л. В. Перекисное окисление мембранных липидов холодостойких растений при повреждении отрицательными температурами // Физиология растений. – 1982, т. 29, вып. 6, с. 1045–1052

9. Жуков А. В., Лебедева Н. И., Верещагін А. Г. Влияние гельминтоспориозной корневой гнили на содержание липидов в проростках пшеницы // Физиология растений. – 2001, т. 48, № 1, с. 132–136

10. Левитин М. М., Тютерев С. Л. Пшеница: гнили корней, корневой шейки и проростков // Защита и карантин растений / Приложение: Грибные болезни зерновых культур. – 2003, № 11, с. 57(5)–59(7)

11. Лукаткин А. С., Голованова В. С. Интенсивность перекисного окисления липидов в охлажденных листьях теплолюбивых растений // Физиология растений. – 1988, т. 35, вып. 4, с. 773–780

12. Мерзляк М. Н., Шевырева В. В., Румянцева В. Б., Жиров В. К. Флюорометрическое определение малонового диальдегида в тканях растений // Научные доклады высшей школы / Биологические науки. – 1979, № 10, с. 70

13. Платонова А. А., Остишин С. С. Вміст малонового діальдегіду та активність антиоксидантних ферментів у проростках гороху за дії іонів кадмію // Физиология и биохимия культурных растений. – 2000, т. 32, № 2, с. 146–150

14. Тютерев С. Л. Краткая характеристика наиболее опасных болезней // Защита и карантин растений / Приложение: Протравливание семян зерновых колосовых культур. – 2001, № 3, с. 91(3)–99(11)

15. Шакирова Ф. М. Неспецифическая устойчивость к стрессовым факторам и ее регуляция. – Уфа, 2001. – 160 с.